12-04-00

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PATENT APPLICATION TRANSMITTAL LETTER	Attorney Docket No.: A-692

To the Assistant Commissioner for Patents:

In re the patent application of: Andrew A. Welcher, Ulla M. Sarmiento, Henry Schultz, Hilary T. Chute

For: B7-LIKE MOLECULES AND USES THEREOF

Transmitted herewith are:

- ☒ 124 pages of specification, 17 pages of claim(s) and 1 pages of abstract, totaling 142 pages.
- ☒ 17 sheet(s) of drawings.
- ☒ a declaration by the applicant(s).
- ☒ 24 pages of sequence listing.
- ☐ a certified copy of _____
- ☒ Other: Attorney Statement pursuant to 37 CFR 1.821; Computer Disk with SEQ. Listing; Recordation Form coversheet with Assignment; Information Disclosure Statement with 1449.
- ☒ **Preliminarily, please amend the specification by inserting before the first line the following:**

--This application claims the benefit of U.S. Provisional Application No. 60/214,512, filed June 28, 2000, which is hereby incorporated by reference.--

CLAIMS AS FILED

For	Number Filed		Number Extra		Rate		Fee
Total Claims	118	- 20 =	98	x	$18.00	=	$1764.00
Independent Claims	11	- 3 =	8	x	$80.00	=	640.00
Multiple Dependent Claims	X			+	$270.00	=	270.00
Basic Fee					$710.00	=	$710.00
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- ☒ Please charge Deposit Account No. 01-0519, in the name of Amgen Inc., in the amount of $3,384.00. An original and one copy are enclosed.
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Respectfully submitted,

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B7-Like Molecules and Uses Thereof

This application claims the benefit of U.S. Provsional Application No. 60/214,512, filed June 28, 2000, which is hereby incorporated by reference

Field of the Invention

The present invention relates to novel B7-like polypeptides and nucleic acid molecules encoding the same. The invention also relates to vectors, host cells, pharmaceutical compositions, selective binding agents and methods for producing B7-like polypeptides. Also provided for are methods for the diagnosis, treatment, amelioration, and/or prevention of diseases associated with B7-like polypeptides.

Background of the Invention

Technical advances in the identification, cloning, expression and manipulation of nucleic acid molecules and the deciphering of the human genome have greatly accelerated the discovery of novel therapeutics. Rapid nucleic acid sequencing techniques can now generate sequence information at unprecedented rates and, coupled with computational analyses, allow the assembly of overlapping sequences into partial and entire genomes and the identification of polypeptide-encoding regions. A comparison of a predicted amino acid sequence against a database compilation of known amino acid sequences allows one to determine the extent of homology to previously identified sequences and/or structural landmarks. The cloning and expression of a polypeptide-encoding region of a nucleic acid molecule provides a polypeptide product for structural and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides may confer

advantageous properties on a product for use as a therapeutic.

In spite of the significant technical advances in genome research over the past decade, the potential for the development of novel therapeutics based on the human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics, or those encoding polypeptides, which may act as "targets" for therapeutic molecules, have still not been identified.

Accordingly, it is an object of the invention to identify novel polypeptides and nucleic acid molecules encoding the same, which have diagnostic or therapeutic benefit.

Summary of the Invention

The present invention relates to novel B7-like nucleic acid molecules and encoded polypeptides.

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5 or 7;

(b) the nucleotide sequence as set forth in SEQ ID NOs: 9, 11 or 13;

(c) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) a nucleotide sequence which hybridizes under

moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14; and

(g) a nucleotide sequence complementary to any of (a)-(f).

The invention also provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99 percent identical to the polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the polypeptide as set forth in SEQ ID NOs: 10, 12 or 14, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(c) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5 or 7, wherein the

encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NOs: 9, 11 or 13, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) a nucleotide sequence of SEQ ID NOs: 1, 3, 5 or 7, or (a) or (b), above, encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) a nucleotide sequence of SEQ ID NOs: 9, 11 or 13, or (a) or (b), above, encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

g) a nucleotide sequence encoding a polypeptide that has a substitution and/or deletion of 1 to 100 amino acid residues as set forth in any of SEQ ID NOs: 9, 11 or 13, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12, or 14;

h) a nucleotide sequence of SEQ ID NOs: 1, 3, 5 or 7, or (a), (c), (e) or (f), above, comprising a fragment of at least about 16 nucleotides;

i) a nucleotide sequence of SEQ ID NOs: 9, 11 or

13, or (b), (d), (f) or (h), above, comprising a fragment of at least about 16 nucleotides;

j) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a), (c), (e), (g) or (i) , above,, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

k) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (b), (d), (f), (h) or (j), above, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14; and

l) a nucleotide sequence complementary to any of (a)-(l).

The invention further provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least

one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(g) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(h) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(i) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one modification selected from the group consisting of

at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(j) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one modification selected from the group consisting of at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(k) a nucleotide sequence of (a)-(j) comprising a fragment of at least about 16 nucleotides;

(l) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a), (c), (e), (g), (i) or (k), wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(m) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (b), (d), (f), (h), (j) or (k), wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;and

(n) a nucleotide sequence complementary to any of (a)-(m).

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 2, and optionally further comprising an amino-terminal methionine;

(b) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 4, and optionally further comprising an amino-terminal methionine;

(c) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 6, and optionally further comprising an amino-terminal methionine;

(d) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 8, and optionally further comprising an amino-terminal methionine;

(e) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 10, and optionally further comprising an amino-terminal methionine;

(f) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 12, and optionally further comprising an amino-terminal methionine;

(g) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 14, and optionally further comprising an amino-terminal methionine;

(h) an amino acid sequence for an ortholog of any one of SEQ ID NOs: 2, 4, 6 or 8, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(i) an amino acid sequence for an ortholog of any one of SEQ ID NOs: 10, 12 or 14, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(j) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(k) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence of SEQ ID NOs: 10, 12 or 14, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(l) a fragment of the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(m) a fragment of the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(n) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8, or at least one of (a), (c), (e), (f), (h), (i), (k) or (l), above, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4,

6 or 8; and

(o) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14, or at least one of (b), (d), (f), (h), (j), (l) or (m) wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14.

The invention further provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(c) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(g) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(h) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(i) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8, with at least one modification selected from the group consisting of at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8; and

(j) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14, with at least one modification selected from the group consisting of at least one amino acid substitution, amino acid insertion, amino

acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14.

Also provided are fusion polypeptides comprising the amino acid sequences of (a)-(j) above.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein, recombinant host cells comprising recombinant nucleic acid molecules as set forth herein, and a method of producing a B7-like polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced.

A transgenic non-human animal comprising a nucleic acid molecule encoding a B7-like polypeptide is also encompassed by the invention. The B7-like nucleic acid molecules are introduced into the animal in a manner that allows expression and increased levels of the B7-like polypeptide, which may include increased circulating levels. The transgenic non-human animal is preferably a mammal.

Also provided are derivatives of the B7-like polypeptides of the present invention.

Additionally provided are selective binding agents such as antibodies and peptides capable of specifically binding the at least one of the B7-like polypeptides of the invention. Such antibodies and peptides may be agonistic or antagonistic.

Pharmaceutical compositions comprising the nucleotides, polypeptides, or selective binding agents of the present invention and one or more

pharmaceutically acceptable formulation agents are also encompassed by the invention. The pharmaceutical compositions are used to provide therapeutically effective amounts of the nucleotides or polypeptides of the present invention. The invention is also directed to methods of using the polypeptides, nucleic acid molecules, and selective binding agents.

The B7-like polypeptides and nucleic acid molecules of the present invention may be used to treat, prevent, ameliorate, and/or detect diseases and disorders, including those recited herein.

The present invention also provides a method of assaying test molecules to identify a test molecule which binds to at least one B7-like polypeptide. The method comprises contacting a B7-like polypeptide with a test molecule and determining the extent of binding of the test molecule to the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists of at least one B7-like polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of at least one B7-like polypeptide or on the activity of the mature form of at least one B7-like polypeptide.

Methods of regulating expression and modulating (*i.e.*, increasing or decreasing) levels of at least one B7-like polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule encoding a B7-like polypeptide. In another method, a nucleic acid molecule comprising elements that regulate or modulate the expression of a B7-like polypeptide may be

administered. Examples of these methods include gene therapy, cell therapy, and anti-sense therapy as further described herein.

In another aspect of the present invention, the B7-like polypeptides may be used for identifying receptors thereof ("B7-like receptors"). Various forms of "expression cloning" have been extensively used for cloning receptors for protein ligands. See for example, H. Simonsen and H.F. Lodish, Trends in Pharmacological Sciences, 15:437-441 (1994), and Tartaglia et al., *Cell*, 83:1263-1271 (1995). The isolation of the B7-like receptor(s) is useful for identifying or developing novel agonists and antagonists of the B7-like polypeptide-signaling pathway. Such agonists and antagonists include soluble B7-like receptor(s), anti-B7-like receptor(s) selective binding agents (such as antibodies and derivatives thereof), small molecules, and antisense oligonucleotides, any of which can be used for treating one or more of the diseases or disorders, including those recited herein.

Brief Description of the Figures

Figure 1 depicts a nucleic acid sequence (SEQ ID NO:1) encoding human B7-like protein (B71.h1). Also depicted is the amino acid sequence (SEQ ID NO:2) of human B7-like protein (B71.h1).

Figure 2 depicts a nucleic acid sequence (SEQ ID NO:3) encoding human B7-like protein (B71.h2). Also depicted is the amino acid sequence (SEQ ID NO:4) of human B7-like protein (B71.h2).

Figure 3 depicts a nucleic acid sequence (SEQ ID NO:5) encoding human B7-like protein (B71.h3). Also depicted is the amino acid sequence (SEQ ID NO:6) of human B7-like protein (B71.h3).

Figure 4 depicts a nucleic acid sequence (SEQ ID NO:7) encoding human B7-like protein (B71.h4). Also depicted is the amino acid sequence (SEQ ID NO:8) of human B7-like protein (B71.h4).

Figure 5 depicts a nucleic acid sequence (SEQ ID NO:9) encoding murine B7-like protein (B71.m1). Also depicted is the amino acid sequence (SEQ ID NO:10) of murine B7-like protein (B71.m1).

Figure 6 depicts a nucleic acid sequence (SEQ ID NO:11) encoding murine B7-like protein B71.m2). Also depicted is the amino acid sequence (SEQ ID NO:12) of murine B7-like protein (B71.m2).

Figure 7 depicts a nucleic acid sequence (SEQ ID NO:13) encoding murine B7-like protein (B71.m3). Also depicted is the amino acid sequence (SEQ ID NO:14) of murine B7-like protein (B71.m3).

Figure 8 depicts a comparison of the amino acid sequences of SEQ ID NO: 8 and rat B7-1 (SEQ ID NO:15).

Figure 9 depicts a comparison of the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO:9.

Detailed Description of the Invention

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein.

Definitions

The terms "B7-like gene(s)" or "B7-like nucleic acid molecule(s)" or "B7-like polynucleotide(s)" refer to one or more nucleic acid molecules comprising or consisting of nucleotide sequences as set forth in SEQ ID NOs: 1, 3, 5 or 7, which encode recombinant human proteins, nucleotide sequences encoding recombinant human polypeptides as set forth in SEQ ID NOs: 2, 4, 6 or 8, nucleotide sequences of the DNA inserts in B71.h1, B71.h2, B71.h3 or B71.h4, and of nucleotide sequences as set forth in SEQ ID NOs: 10, 12 or 14, which encode recombinant murine proteins, nucleotide sequences encoding recombinant murine polypeptides as set forth in SEQ ID NOs: 10, 12 or 14, and nucleic acid molecules as defined herein.

The term "B7-like polypeptide(s)" refers to one or more recombinant human polypeptides comprising the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8, related polypeptides thereto, or recombinant murine polypeptides comprising the amino acid sequence of SEQ ID NOs: 10, 12 or 14, and related polypeptides thereto. Related polypeptides include: B7-like polypeptide allelic variants, B7-like polypeptide orthologs, B7-like polypeptide splice variants, B7-like polypeptide variants and B7-like polypeptide derivatives. B7-like polypeptides may be mature polypeptides, as defined

herein, and may or may not have an amino terminal methionine residue, depending on the method by which they are prepared.

The term "B7-like polypeptide allelic variant(s)" refers to one or more of several possible naturally occurring alternate forms of a gene occupying a given locus on a chromosome of an organism or a population of organisms.

The term "B7-like polypeptide derivative(s)" refers to one or more of the polypeptides as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14, B7-like polypeptide allelic variants, B7-like polypeptide orthologs, B7-like polypeptide splice variants, or B7-like polypeptide variants, as defined herein, that have been chemically modified.

The term "B7-like polypeptide fragment(s)" refers to one or more polypeptides that comprise a truncation at the amino terminus (with or without a leader sequence) and/or a truncation at the carboxy terminus of the polypeptides as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14, B7-like polypeptide allelic variants, B7-like polypeptide orthologs, B7-like polypeptide splice variants and/or a B7-like polypeptide variant having one or more amino acid additions or substitutions or internal deletions (wherein the resulting polypeptide is at least 6 amino acids or more in length) as compared to the B7-like polypeptide amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. B7-like polypeptide fragments may result from alternative RNA splicing or from *in vivo* protease activity. In preferred embodiments, truncations comprise about 10 amino acids, or about 20 amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or more than about 100 amino acids. The polypeptide fragments

so produced will comprise about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 150 amino acids, or about 200 amino acids. Such B7-like polypeptide fragments may optionally comprise an amino terminal methionine residue. It will be appreciated that such fragments can be used, for example, to generate antibodies to B7-like polypeptides.

The term "B7-like fusion polypeptide(s)" refers to fusions of one or more amino acids (such as a heterologous peptide or polypeptide) at the amino or carboxy terminus of one or more of the polypeptides set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12 or 14, B7-like polypeptide allelic variants, B7-like polypeptide orthologs, B7-like polypeptide splice variants, or B7-like polypeptide variants having one or more amino acid deletions, substitutions or internal additions as compared to a B7-like polypeptide amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

The term "B7-like polypeptide ortholog(s)" refers to one or more polypeptides from other species that correspond to the amino acid sequences as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. For example, mouse and human B7-like polypeptides are considered orthologs of each other.

The term "B7-like polypeptide splice variant(s)" refers to one ore more nucleic acid molecules, usually RNA, which are generated by alternative processing of intron sequences in an RNA transcript of the B7-like polypeptide amino acid sequences as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

The term "B7-like polypeptide variant(s)" refers to B7-like polypeptides comprising amino acid sequences

having one or more amino acid sequence substitutions, deletions (such as internal deletions and/or B7-like polypeptide fragments), and/or additions (such as internal additions and/or B7-like fusion polypeptides) as compared to the B7-like polypeptide amino acid sequence set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 (with or without a leader sequence). Variants may be naturally occurring (*e.g.*, B7-like polypeptide allelic variants, B7-like polypeptide orthologs and B7-like polypeptide splice variants) or may be artificially constructed. Such B7-like polypeptide variants may be prepared from the corresponding nucleic acid molecules having a DNA sequence that varies accordingly from the DNA sequence as set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13. In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, wherein the substitutions may be conservative, or non-conservative, or any combination thereof.

The term "antigen(s)" refers to one or more molecules or portion thereof capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

The term "biologically active B7-like polypeptide(s)" refers to one or more B7-like polypeptides having at least one activity

characteristic of a polypeptide comprising the amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

The terms "effective amount" and "therapeutically effective amount" each refer to the amount of a B7-like polypeptide or B7-like nucleic acid molecule used to support an observable level of one or more biological activities of the B7-like polypeptides as set forth herein.

The term "expression vector" refers to a vector which is suitable for use in a host cell and contains nucleic acid sequences which direct and/or control the expression of heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

The term "identity" as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two

or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*i.e.*, "algorithms").

The term "similarity" is a related concept, but in contrast to "identity", refers to a measure of similarity which includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are 5 more positions where there are conservative substitutions, then the percent identity remains 50%, but the per cent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the degree of similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the invention that (1) has been separated from at least about 50 percent of proteins, lipids, carbohydrates or other materials with which it is naturally found when total DNA is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other

contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates or other materials with which it is naturally found when isolated from the source cell, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "mature B7-like polypeptide(s)" refers to one or more B7-like polypeptide lacking a leader sequence. A mature B7-like polypeptide may also include other modifications such as proteolytic processing of the amino terminus (with or without a leader sequence) and/or the carboxy terminus, cleavage of a smaller polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and the like.

The term "nucleic acid sequence(s)" or "nucleic acid molecule(s)" refers to one or more DNA or RNA sequences. The term encompasses molecules formed from

any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-cytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxy-methylaminomethyluracil, dihydrouracil, inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5' -methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the

flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "pharmaceutically acceptable carrier(s)" or "physiologically acceptable carrier(s)" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of the B7-like polypeptide, B7-like nucleic acid molecule or B7-like selective binding agent as a pharmaceutical composition.

The term "selective binding agent(s)" refers to a molecule or molecules having specificity for a B7-like polypeptide. As used herein, the terms, "specific" and "specificity" refer to the ability of the selective binding agents to bind to human B7-like polypeptides and not to bind to human non-B7-like polypeptides. It will be appreciated, however, that the selective binding agents may also bind orthologs of the polypeptides as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14, that is, interspecies versions thereof,

such as mouse and rat polypeptides.

The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the acquisition and transfer of eukaryotic cellular sequences by retroviruses.

The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, for example, Graham *et al.*, *Virology*, 52:456 (1973); Sambrook *et al.*, *Molecular Cloning, a laboratory Manual*, Cold Spring Harbor Laboratories New York (1989); Davis *et al.*, *Basic Methods in Molecular Biology*, Elsevier (1986); and Chu *et al.*, *Gene*, 13:197 (1981). Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A cell is considered to

have been stably transformed when the DNA is replicated with the division of the cell.

The term "vector" is used to refer to any molecule (*e.g.*, nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

Relatedness of Nucleic Acid Molecules and/or Polypeptides

It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecules of SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13, and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising or consisting essentially of a substitution, modification, addition and/or a deletion of one or more amino acid residues compared to the polypeptides in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

Fragments include molecules which encode a polypeptide of at least about 25 amino acid residues, or about 50, or about 75, or about 100, or greater than about 100 amino acid residues of the polypeptides of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

In addition, related B7-like nucleic acid molecules include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully complementary sequence of the nucleic acid molecules of SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13, or of a molecule encoding a polypeptide, which polypeptide

comprises the amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14, or of a nucleic acid fragment as defined herein, or of a nucleic acid fragment encoding a polypeptide as defined herein. Hybridization probes may be prepared using the B7-like sequences provided herein to screen cDNA, genomic or synthetic DNA libraries for related sequences. Regions of the DNA and/or amino acid sequence of B7-like polypeptides that exhibit significant identity to known sequences are readily determined using sequence alignment algorithms as described herein, and those regions may be used to design probes for screening.

The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of "highly stringent conditions" for hybridization and washing are 0.015M sodium chloride, 0.0015M sodium citrate at 65-68°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989); Anderson *et al.*, Nucleic Acid Hybridisation: a practical approach, Ch. 4, IRL Press Limited (Oxford, England).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the

rate of hybridization will be affected. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate ($NaDodSO_4$ or SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson *et al.*, Nucleic Acid Hybridisation: a Practical Approach, Ch. 4, IRL Press Limited (Oxford, England).

Factors affecting the stability of a DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

$$T_m(°C) = 81.5 + 16.6(\log[Na+]) + 0.41(\%G+C) - 600/N - 0.72(\%formamide)$$

where N is the length of the duplex formed, [Na+] is the molar concentration of the sodium ion in the hybridization or washing solution, %G+C is the percentage of (guanine+cytosine) bases in the hybrid.

For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015M sodium chloride, 0.0015M sodium citrate at 50-65°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 20% formamide at 37-50°C. By way of example, a "moderately stringent" condition of 50°C in 0.015 M sodium ion will allow about a 21% mismatch.

It will be appreciated by those skilled in the art that there is no absolute distinction between "highly" and "moderately" stringent conditions. For example, at 0.015M sodium ion (no formamide), the melting temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences, one skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1M NaCl* for oligonucleotide probes up to about 20nt is given by:

Tm = 2°C per A-T base pair + 4°C per G-C base pair

*The sodium ion concentration in 6X salt sodium citrate (SSC) is 1M. See Suggs *et al.*, Developmental

Biology Using Purified Genes, p. 683, Brown and Fox (eds.) (1981).

High stringency washing conditions for oligonucleotides are usually at a temperature of 0-5°C below the Tm of the oligonucleotide in 6X SSC, 0.1% SDS.

In another embodiment, related nucleic acid molecules comprise or consist of a nucleotide sequence that is about 70 percent identical to the nucleotide sequences as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13, or comprise or consist essentially of a nucleotide sequence encoding a polypeptide that is about 70 percent identical to the polypeptides as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. In preferred embodiments, the nucleotide sequences are about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequences as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13, or the nucleotide sequences encode a polypeptide that is about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the polypeptide sequences as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

Differences in the nucleic acid sequence may result in conservative and/or non-conservative modifications of an amino acid sequence relative to an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

Conservative modifications to an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 (and

the corresponding modifications to the encoding nucleotides) will produce B7-like polypeptides having functional and chemical characteristics similar to those of naturally occurring B7-like polypeptides. In contrast, substantial modifications in the functional and/or chemical characteristics of B7-like polypeptides may be accomplished by selecting substitutions in an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the B7-like polypeptides, or to increase or decrease the affinity of the B7-like polypeptides described herein.

Exemplary amino acid substitutions are set forth in Table I.

Table I

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues

which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
3) acidic: Asp, Glu;
4) basic: His, Lys, Arg;
5) residues that influence chain orientation: Gly, Pro; and
6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human B7-like polypeptides that are homologous with non-human B7-like polypeptide orthologs, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9);

tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte *et al.*, *J. Mol. Biol.*, 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1);

alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 using well-known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a B7-like polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a B7-like polypeptide that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of such B7-like polypeptide. One skilled in the art would also know that, even in

relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in the B7-like polypeptides that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of B7-like polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a B7-like polypeptide with respect to its three-dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid

substitution at each desired amino acid residue. The variants can then be screened using activity assays know to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., *Curr. Op. in Biotech.*, 7(4):422-427 (1996), Chou *et al.*, *Biochemistry*, 13(2):222-245 (1974); Chou *et al.*, *Biochemistry*, 113(2):211-222 (1974); Chou *et al.*, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou *et al.*, *Ann. Rev. Biochem.*, 47:251-276 and Chou *et al.*, *Biophys. J.*, 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's *or* protein's structure. *See* Holm *et al.*,

Nucl. Acid. Res., 27(1):244-247 (1999). It has been suggested (Brenner *et al.*, *Curr. Op. Struct. Biol.*, 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide *or* protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

Additional methods of predicting secondary structure include "threading" (Jones, D., *Curr. Opin. Struct. Biol.*, 7(3):377-387 (1997); Sippl *et al.*, *Structure*, 4(1):15-9 (1996)), "profile analysis" (Bowie *et al.*, *Science*, 253:164-170 (1991); Gribskov *et al.*, *Meth. Enzym.*, 183:146-159 (1990); Gribskov *et al.*, *Proc. Nat. Acad. Sci.*, 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Holm, *supra*, and Brenner, *supra*).

Preferred B7-like polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites have been altered compared to a amino acid sequence set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. In one embodiment, B7-like polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites than an amino acid sequence set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. An N-linked glycosylation site is characterized by the sequence Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution(s) of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove

an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred B7-like variants include cysteine variants, wherein one or more cysteine residues are deleted from or substituted for another amino acid (*e.g.*, serine) as compared to an amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14. Cysteine variants are useful when B7-like polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

Figure 8 depicts a topological comparison of the amino acid sequences of SEQ ID NO:10 and rat B7-1 (SEQ ID NO:15). Figure 9 depicts a topological comparison of the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO:10. A skilled artisan can readily discern the residues conserved among the family members and, with this information, can readily generate variants within the scope of this invention, using standard techniques.

In addition, a polypeptide comprising the amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12, or 14 or a B7-like polypeptide variant may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection

and/or isolation of a B7-like fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain, or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the polypeptide comprising an amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 or a B7-like polypeptide variant. Specific fusions may include the fusion of one or more of SEQ ID Nos: 2, 4, 6, 8, 10, 12 or 14.

Fusions can be made either at the amino terminus or at the carboxy terminus of the polypeptide comprising an amino acid sequence set forth in SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 or a B7-like polypeptide variant. Fusions may be direct with no linker or adapter molecule or indirect using a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically up to about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

In a further embodiment of the invention, the polypeptide comprising an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 or a B7-like polypeptide variant is fused to one or more domains of an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab", which binds antigen, and a constant domain known as "Fc", which is involved in effector functions such as complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas an Fab is short-lived. Capon *et al.*, *Nature*, 337:525-531 (1989). When constructed together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein A binding, complement fixation and perhaps even placental transfer. *Id.* Table II summarizes the use of certain Fc fusions known in the art.

Table II
Fc Fusion with Therapeutic Proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fcγ2a	IL-10	anti-inflammatory; transplant rejection	Zheng *et al.* (1995), *J. Immunol.*, 154: 5590-5600
IgG1	TNF receptor	septic shock	Fisher *et al.* (1996), *N. Engl. J. Med.*, 334: 1697-1702; Van Zee *et al.*, (1996), *J. Immunol.*, 156: 2221-2230
IgG, IgA,	TNF	inflammation,	U.S. Pat. No.

IgM, or IgE (excluding the first domain)	receptor	autoimmune disorders	5,808,029, issued September 15, 1998
IgG1	CD4 receptor	AIDS	Capon *et al.* (1989), *Nature* 337: 525-531
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill *et al.* (1995), *Immunotech.*, 1: 95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614, published July 3, 1997
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig Cγ1	CTLA-4	autoimmune disorders	Linsley (1991), *J. Exp. Med.*, 174:561-569

In one example, all or a portion of the human IgG hinge, CH2 and CH3 regions may be fused at either the N-terminus or C-terminus of the B7-like polypeptides using methods known to the skilled artisan. The resulting B7-like fusion polypeptide may be purified by use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, reduce aggregation, etc.

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular

Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo *et al.*, *SIAM J. Applied Math.*, 48:1073 (1988).

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, *Nucl. Acid. Res.*, 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, *J. Mol. Biol.*, 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (*BLAST Manual,* Altschul *et al.* NCB/NLM/NIH Bethesda, MD 20894; Altschul *et al., supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship

between the two full-length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the target polypeptide.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff *et al.*, *Atlas of Protein Sequence and Structure*, vol. 5, supp.3 (1978) for the PAM 250 comparison matrix; Henikoff *et al.*, *Proc. Natl. Acad. Sci USA*, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

Preferred parameters for a polypeptide sequence comparison include the following:

Algorithm: Needleman *et al.*, *J. Mol. Biol.*, 48:443-453 (1970);

Comparison matrix: BLOSUM 62 from Henikoff *et al.*, *Proc. Natl. Acad. Sci.* USA, 89:10915-10919 (1992);
Gap Penalty: 12
Gap Length Penalty: 4
Threshold of Similarity: 0

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparisons include the following:

Algorithm: Needleman *et al.*, *J. Mol Biol.*, 48:443-453 (1970);
Comparison matrix: matches = +10, mismatch = 0
Gap Penalty: 50
Gap Length Penalty: 3

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA to DNA, protein to protein,

protein to DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

Synthesis

It will be appreciated by those skilled in the art that the nucleic acid and polypeptide molecules described herein may be produced by recombinant and other means.

Nucleic Acid Molecules

The nucleic acid molecules encoding a polypeptide comprising the amino acid sequence of a B7-like polypeptide can readily be obtained in a variety of ways including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening and/or PCR amplification of cDNA.

Recombinant DNA methods used herein are generally those set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and/or Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, NY (1994). The present invention provides for nucleic acid molecules as described herein and methods for obtaining the molecules.

Where a gene encoding the amino acid sequence of a B7-like polypeptide has been identified from one species, all or a portion of that gene may be used as a

probe to identify orthologs or related genes from the same species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express a B7-like polypeptide. In addition, part or all of a nucleic acid molecule having the sequences as set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11 or 13 may be used to screen a genomic library to identify and isolate a gene encoding the amino acid sequence of a B7-like polypeptide. Typically, conditions of moderate or high stringency will be employed for screening to minimize the number of false positives obtained from the screen.

Nucleic acid molecules encoding the amino acid sequence of B7-like polypeptides may also be identified by expression cloning which employs the detection of positive clones based upon a property of the expressed protein. Typically, nucleic acid libraries are screened by the binding of an antibody or other binding partner (*e.g.*, receptor or ligand) to cloned proteins which are expressed and displayed on a host cell surface. The antibody or binding partner is modified with a detectable label to identify those cells expressing the desired clone.

Recombinant expression techniques conducted in accordance with the descriptions set forth below may be followed to produce these polynucleotides and to express the encoded polypeptides. For example, by inserting a nucleic acid sequence which encodes the amino acid sequence of a B7-like polypeptide into an appropriate vector, one skilled in the art can readily produce large quantities of the desired nucleotide sequence. The sequences can then be used to generate

detection probes or amplification primers. Alternatively, a polynucleotide encoding the amino acid sequence of a B7-like polypeptide can be inserted into an expression vector. By introducing the expression vector into an appropriate host, an encoded B7-like polypeptide may be produced in large amounts.

Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically complementary to two separate regions of cDNA (oligonucleotides) encoding the amino acid sequence of a B7-like polypeptide, are then added to the cDNA along with a polymerase such as *Taq* polymerase, and the polymerase amplifies the cDNA region between the two primers.

Another means of preparing a nucleic acid molecule encoding the amino acid sequence of a B7-like polypeptide is chemical synthesis using methods well known to the skilled artisan such as those described by Engels *et al., Angew. Chem. Intl. Ed.,* 28:716-734 (1989). These methods include, *inter alia,* the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the amino acid sequence of a B7-like polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full-length nucleotide sequence of

a B7-like polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of a B7-like polypeptide, depending on whether the polypeptide produced in the host cell is designed to be secreted from that cell. Other methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have been altered for the optimal expression of a B7-like polypeptide in a given host cell. Particular codon alterations will depend upon the B7-like polypeptide(s) and host cell(s) selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host cell. Computer algorithms which incorporate codon frequency tables such as "Ecohigh.cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, WI. Other useful codon frequency tables include "Celegans_high.cod", "Celegans_low.cod", "Drosophila_high.cod", "Human_high.cod", "Maize_high.cod", and "Yeast_high.cod".

Vectors and Host Cells

A nucleic acid molecule encoding the amino acid sequence of a B7-like polypeptide may be inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed

(*i.e.*, the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). A nucleic acid molecule encoding the amino acid sequence of a B7-like polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems), and/or eukaryotic host cells. Selection of the host cell will depend in part on whether a B7-like polypeptide is to be post-translationally modified (*e.g.*, glycosylated and/or phosphorylated). If so, yeast, insect or mammalian host cells are preferable. For a review of expression vectors, see *Meth. Enz.*, v.185, D.V. Goeddel, ed. Academic Press Inc., San Diego, CA (1990).

Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

Optionally, the vector may contain a "tag"-encoding sequence, *i.e.*, an oligonucleotide molecule located at the 5' or 3' end of a B7-like polypeptide

coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or other "tag" such as FLAG, HA (hemaglutinin Influenza virus) or *myc* for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of a B7-like polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from a purified B7-like polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source) or synthetic, or the flanking sequences may be native sequences which normally function to regulate B7-like polypeptide expression. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence is functional in, and can be activated by, the host cell machinery.

The flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein other than B7-like gene flanking sequences will have been previously identified by mapping and/or by restriction endonuclease digestion

and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with suitable oligonucleotide and/or flanking sequence fragments from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification, Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of a B7-like polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence and ligated into the vector. For example, the origin of replication from the plasmid pBR322 (Product No. 303-3s, New

England Biolabs, Beverly, MA) is suitable for most Gram-negative bacteria and various origins (*e.g.*, SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV) or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene which will be expressed. Amplification is the process wherein genes which are in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure which only the transformants are uniquely adapted to survive by virtue of the selection gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to the amplification of both the selection gene and the DNA that encodes a B7-like polypeptide. As a result, increased quantities of a B7-like polypeptide are synthesized from the amplified DNA.

A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of a B7-like polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth herein and used in a prokaryotic vector.

A leader, or signal, sequence may be used to direct a B7-like polypeptide out of the host cell.

Typically, a nucleotide sequence encoding the signal sequence is positioned in the coding region of a B7-like nucleic acid molecule, or directly at the 5' end of a B7-like polypeptide coding region. Many signal sequences have been identified, and any of those that are functional in the selected host cell may be used in conjunction with a B7-like nucleic acid molecule. Therefore, a signal sequence may be homologous (naturally occurring) or heterologous to a B7-like gene or cDNA. Additionally, a signal sequence may be chemically synthesized using methods described herein. In most cases, the secretion of a B7-like polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the secreted B7-like polypeptide. The signal sequence may be a component of the vector, or it may be a part of a B7-like nucleic acid molecule that is inserted into the vector.

Included within the scope of this invention is the use of either a nucleotide sequence encoding a native B7-like polypeptide signal sequence joined to a B7-like polypeptide coding region or a nucleotide sequence encoding a heterologous signal sequence joined to a B7-like polypeptide coding region. The heterologous signal sequence selected should be one that is recognized and processed, *i.e.*, cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native B7-like polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or heat-stable enterotoxin II leaders. For yeast secretion, the native B7-like

polypeptide signal sequence(s) may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add presequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the N-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired B7-like polypeptide, if the enzyme cuts at such area within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule is increased by the presence of one or more introns in the vector; this is particularly true where a polypeptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within a B7-like gene, especially where the gene used is a full-length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron(s) may be obtained from another source. The position of the intron with respect to

flanking sequences and a B7-like gene is generally important, as the intron must be transcribed to be effective. Thus, when a B7-like cDNA molecule is being transcribed, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA transcription termination sequence. Preferably, the intron or introns will be located on one side or the other (*i.e.*, 5' or 3') of the cDNA such that it does not interrupt the coding sequence. Any intron from any source, including any viral, prokaryotic and eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell(s) into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

The expression and cloning vectors of the present invention will each typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding a B7-like polypeptide. Promoters are untranscribed sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription of the structural gene. Promoters are conventionally grouped into one of two classes, inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, initiate continual gene product production; that is, there is little or no control over gene expression. A large number of promoters, recognized by a variety of

potential host cells, are well known. A suitable promoter is operably linked to the DNA encoding a B7-like polypeptide by removing the promoter from the source DNA by restriction enzyme digestion and inserting the desired promoter sequence into the vector. The native B7-like gene promoter sequence(s) may be used to direct amplification and/or expression of a B7-like nucleic acid molecule. A heterologous promoter is preferred, however, if it permits greater transcription and higher yields of the expressed protein as compared to the native promoter, and if it is compatible with the host cell system that has been selected for use.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase, a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence(s), using linkers or adapters as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (CMV), a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40).

Other suitable mammalian promoters include heterologous mammalian promoters, *e.g.*, heat-shock promoters and the actin promoter.

Additional promoters which may be of interest in controlling B7-like gene transcription include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, *Nature*, 290:304-310 (1981)); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, *Cell*, 22:787-797 (1980)); the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. USA*, 78:144-1445 (1981)); the regulatory sequences of the metallothionine gene (Brinster *et al.*, *Nature*, 296:39-42 (1982)); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff, *et al.*, *Proc. Natl. Acad. Sci. USA*, 75:3727-3731 (1978)); or the tac promoter (DeBoer, *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell*, 38:639-646 (1984); Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 50:399-409 (1986); MacDonald, *Hepatology*, 7:425-515 (1987)); the insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature*, 315:115-122 (1985)); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell*, 38:647-658 (1984); Adames *et al.*, *Nature*, 318:533-538 (1985); Alexander *et al.*, *Mol. Cell. Biol.*, 7:1436-1444 (1987)); the mouse mammary tumor virus control region

which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell*, 45:485-495 (1986)); the albumin gene control region which is active in liver (Pinkert *et al.*, *Genes and Devel.*, 1:268-276 (1987)); the alphafetoprotein gene control region which is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol.*, 5:1639-1648 (1985); Hammer *et al.*, *Science*, 235:53-58 (1987))); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, *Genes and Devel.*, 1:161-171 (1987)); the beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, *Nature*, 315:338-340 (1985); Kollias *et al.*, *Cell*, 46:89-94 (1986)); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, *Cell*, 48:703-712 (1987)); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature*, 314:283-286 (1985)); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, Science, 234:1372-1378 (1986)).

An enhancer sequence may be inserted into the vector to increase the transcription of a DNA encoding an B7-like polypeptide of the present invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent. They have been found 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (*e.g.*, globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The SV40 enhancer, the cytomegalovirus early promoter enhancer,

the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5′ or 3′ to a B7-like nucleic acid molecule, it is typically located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the desired flanking sequences are not already present in the vector, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, Carlsbad, CA), pBSII (Stratagene Company, La Jolla, CA), pET15• (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids, plasmids or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc.,

La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (*e.g.*, TOPO™ TA Cloning® Kit, PCR2.1® plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast, or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA).

After the vector has been constructed and a nucleic acid molecule encoding a B7-like polypeptide has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. The transformation of an expression vector for a B7-like polypeptide into a selected host cell may be accomplished by well known methods including methods such as transfection, infection, calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell or a vertebrate cell). The host cell, when cultured under appropriate conditions, synthesizes a B7-like polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as desired expression levels, polypeptide modifications that are

desirable or necessary for activity, such as glycosylation or phosphorylation, and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. Examples include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR- cells (Urlaub *et al., Proc. Natl. Acad. Sci. USA,* 97:4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), or 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the ATCC. Each of these cell lines is known by and available to those skilled in the art of protein expression.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (*e.g.*, HB101, (ATCC No. 33694) DH5α, DH10, and MC1061 (ATCC No. 53338)) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris*.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts *et al.*, *Biotechniques*, 14:810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, 4:564-572 (1993); and Lucklow *et al.* (*J. Virol.*, 67:4566-4579 (1993). Preferred insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

One may also use transgenic animals to express glycosylated B7-like polypeptides. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide in the animal milk. One may also use plants to produce B7-like polypeptides, however, in general, the glycosylation occurring in plants is different from that produced in mammalian cells, and may result in a glycosylated product which is not suitable for human therapeutic use.

Polypeptide Production

Host cells comprising a B7-like polypeptide expression vector may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells include, for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells include Roswell Park Memorial Institute medium 1640 (RPMI 1640), Minimal Essential Medium (MEM) and/or Dulbecco's Modified Eagle Medium (DMEM), all of which may be supplemented with serum and/or growth factors as indicated by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate and/or fetal calf serum, as necessary.

Typically, an antibiotic or other compound useful for selective growth of transformed cells is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline, and neomycin.

The amount of a B7-like polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis,

HPLC separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If a B7-like polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. If however, a B7-like polypeptide is not secreted from the host cells, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells).

For a B7-like polypeptide situated in the host cell cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells), intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

If a B7-like polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material can then be treated at pH extremes or with a chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. A B7-like polypeptide in its now soluble form can then be analyzed using gel electrophoresis, immunoprecipitation

or the like. If it is desired to isolate a B7-like polypeptide, isolation may be accomplished using standard methods such as those described herein and in Marston *et al.*, *Meth. Enz.*, 182:264-275 (1990).

In some cases, a B7-like polypeptide may not be biologically active upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/ dithiane DTT, and 2-2mercaptoethanol(bME)/dithio-b(ME). A cosolvent may be used to increase the efficiency of the refolding, and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

If inclusion bodies are not formed to a significant degree upon expression of a B7-like polypeptide, then the polypeptide will be found

primarily in the supernatant after centrifugation of the cell homogenate. The polypeptide may be further isolated from the supernatant using methods such as those described herein.

The purification of a B7-like polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (B7-like polypeptide/hexaHis) or another small peptide such as FLAG (Eastman Kodak Co., New Haven, CT) or *myc* (Invitrogen, Carlsbad, CA) at either its carboxyl or amino terminus, it may be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of a B7-like polypeptide/polyHis. See for example, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Section 10.11.8, John Wiley & Sons, New York (1993).

Additionally, a B7-like polypeptide may be purified through the use of a monoclonal antibody which is capable of specifically recognizing and binding to a B7-like polypeptide.

Suitable procedures for purification thus include, without limitation, affinity chromatography, immunoaffinity chromatography, ion exchange chromatography, molecular sieve chromatography, High Performance Liquid Chromatography (HPLC),

electrophoresis (including native gel electrophoresis) followed by gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific, San Francisco, CA). In some cases, two or more purification techniques may be combined to achieve increased purity.

B7-like polypeptides may also be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art, such as those set forth by Merrifield *et al.*, *J. Am. Chem. Soc.*, 85:2149 (1963), Houghten *et al.*, *Proc Natl Acad. Sci. USA*, 82:5132 (1985), and Stewart and Young, Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL (1984). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized B7-like polypeptides may be oxidized using methods set forth in these references to form disulfide bridges. Chemically synthesized B7-like polypeptides are expected to have comparable biological activity to the corresponding B7-like polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with a recombinant or natural B7-like polypeptide.

Another means of obtaining a B7-like polypeptide is via purification from biological samples such as source tissues and/or fluids in which a B7-like polypeptide is naturally found. Such purification can be conducted using methods for protein purification as described herein. The presence of a B7-like polypeptide during purification may be monitored using, for example, an antibody prepared against a recombinantly produced B7-like polypeptide or peptide

fragments thereof.

A number of additional methods for producing nucleic acids and polypeptides are known in the art, and can be used to produce polypeptides having specificity for a B7-like polypeptide. See for example, Roberts *et al.*, *Proc. Natl. Acad. Sci.*, 94:12297-12303 (1997), which describes the production of fusion proteins between an mRNA and its encoded peptide. See also Roberts, R., *Curr. Opin. Chem. Biol.*, 3:268-273 (1999). Additionally, U.S. Patent No. 5,824,469 describes methods of obtaining oligonucleotides capable of carrying out a specific biological function. The procedure involves generating a heterogeneous pool of oligonucleotides, each having a 5′ randomized sequence, a central preselected sequence, and a 3′ randomized sequence. The resulting heterogeneous pool is introduced into a population of cells that do not exhibit the desired biological function. Subpopulations of the cells are then screened for those which exhibit a predetermined biological function. From that subpopulation, oligonucleotides capable of carrying out the desired biological function are isolated.

U.S. Patent Nos. 5,723,323, 5,763,192, 5,814,476 and 5,817,483 describe processes for producing peptides or polypeptides. This is done by producing stochastic genes or fragments thereof, and then introducing these genes into host cells which produce one or more proteins encoded by the stochastic genes. The host cells are then screened to identify those clones producing peptides or polypeptides having the desired activity.

Chemical Derivatives

Chemically modified derivatives of B7-like polypeptides may be prepared by one skilled in the art, given the disclosures set forth hereinbelow. B7-like polypeptide derivatives are modified in a manner that is different, either in the type or location of the molecules naturally attached to the polypeptide. Derivatives may include molecules formed by the deletion of one or more naturally-attached chemical groups. A polypeptide comprising an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14, or a B7-like polypeptide variant may be modified by the covalent attachment of one or more polymers. For example, the polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Included within the scope of suitable polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

The polymers each may be of any molecular weight and may be branched or unbranched. The polymers each typically have an average molecular weight of between about 2kDa to about 100kDa (the term "about" indicating that in preparations of a water soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer preferably is between about 5kDa and about 50kDa, more preferably between about 12kDa and about 40kDa and most preferably between about 20kDa and about 35kDa.

Suitable water soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C_1-C_{10}) alkoxy- or aryloxy-polyethylene glycol), monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran, of, for example about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol) and polyvinyl alcohol. Also encompassed by the present invention are bifunctional crosslinking molecules which may be used to prepare covalently attached multimers of a polypeptide comprising an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14 or a B7-like polypeptide variant.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for preparing chemical derivatives of polypeptides will generally comprise the steps of (a) reacting the polypeptide with the activated polymer molecule (such as a reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby a polypeptide comprising the amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14, or a B7-like polypeptide variant becomes attached to one or more polymer molecules, and (b) obtaining the reaction product(s). The optimal reaction conditions will be determined based on known parameters and the desired result. For example, the larger the ratio of polymer

molecules:protein, the greater the percentage of attached polymer molecule. In one embodiment, a B7-like polypeptide derivative may have a single polymer molecule moiety at the amino terminus. See, for example, U.S. Patent No. 5,234,784.

The pegylation of the polypeptide specifically may be carried out by any of the pegylation reactions known in the art, as described for example in the following references: Francis *et al.*, *Focus on Growth Factors*, 3:4-10 (1992); EP 0154316; EP 0401384 and U.S. Patent No. 4,179,337. For example, pegylation may be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, the polymer(s) selected should have a single reactive ester group. For reductive alkylation, the polymer(s) selected should have a single reactive aldehyde group. A reactive aldehyde is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C_1-C_{10} alkoxy or aryloxy derivatives thereof (see U.S. Patent No. 5,252,714).

In another embodiment, B7-like polypeptides may be chemically coupled to biotin, and the biotin/B7-like polypeptide molecules which are conjugated are then allowed to bind to avidin, resulting in tetravalent avidin/biotin/B7-like polypeptide molecules. B7-like polypeptides may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or

anti-TNP-IgM to form decameric conjugates with a valency of 10.

Generally, conditions which may be alleviated or modulated by the administration of the present B7-like polypeptide derivatives include those described herein for B7-like polypeptides. However, the B7-like polypeptide derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to the non-derivatized molecules.

Genetically Engineered Non-Human Animals

Additionally included within the scope of the present invention are non-human animals such as mice, rats, or other rodents, rabbits, goats, or sheep, or other farm animals, in which the genes encoding the native B7-like polypeptides have been disrupted ("knocked out") such that the level of expression of this gene or genes is (are) significantly decreased or completely abolished. Such animals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032.

The present invention further includes non-human animals such as mice, rats, or other rodents, rabbits, goats, sheep, or other farm animals, in which either the native form of the B7-like genes for that animal or a heterologous B7-like genes are over-expressed by the animal, thereby creating a "transgenic" animal. Such transgenic animals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT application No. WO94/28122.

The present invention further includes non-human animals in which the promoter for one or more of the B7-like polypeptides of the present invention is either activated or inactivated (*e.g.*, by using homologous recombination methods) to alter the level of expression of one or more of the native B7-like polypeptides.

These non-human animals may be used for drug candidate screening. In such screening, the impact of a drug candidate on the animal may be measured. For example, drug candidates may decrease or increase the expression of a B7-like gene. In certain embodiments, the amount of a B7-like polypeptide that is produced may be measured after the exposure of the animal to the drug candidate. Additionally, in certain embodiments, one may detect the actual impact of the drug candidate on the animal. For example, the overexpression of a particular gene may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease expression of the gene or its ability to prevent or inhibit a pathological condition. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product or its ability to prevent or inhibit a pathological condition.

Microarray

It will be appreciated that DNA microarray technology can be utilized in accordance with the present invention. DNA microarrays are miniature, high

density arrays of nucleic acids positioned on a solid support, such as glass. Each cell or element within the array has numerous copies of a single species of DNA which acts as a target for hybridization for its cognate mRNA. In expression profiling using DNA microarray technology, mRNA is first extracted from a cell or tissue sample and then converted enzymatically to fluorescently labeled cDNA. This material is hybridized to the microarray and unbound cDNA is removed by washing. The expression of discrete genes represented on the array is then visualized by quantitating the amount of labeled cDNA which is specifically bound to each target DNA. In this way, the expression of thousands of genes can be quantitated in a high throughput, parallel manner from a single sample of biological material.

This high throughput expression profiling has a broad range of applications with respect to the B7-like molecules of the invention, including, but not limited to: the identification and validation of B7-like genes in disease and as targets for therapeutics; molecular toxicology of B7-like molecules and inhibitors thereof; stratification of populations and generation of surrogate markers for clinical trials; and enhancing B7-like small molecule drug discovery by aiding in the identification of selective compounds in high throughput screens (HTS).

Selective Binding Agents

As used herein, the term "selective binding agent" refers to a molecule which has specificity for one or more B7-like polypeptides. Suitable selective binding

agents include, but are not limited to, antibodies and derivatives thereof, polypeptides, and small molecules. Suitable selective binding agents may be prepared using methods known in the art. An exemplary B7-like polypeptide selective binding agent of the present invention is capable of binding a certain portion of at least one B7-like polypeptide, thereby inhibiting the binding of such polypeptide to the B7-like polypeptide receptor(s).

Selective binding agents such as antibodies and antibody fragments that each bind at least one B7-like polypeptide are within the scope of the present invention. The antibodies may be polyclonal including monospecific polyclonal, monoclonal (MAbs), recombinant, chimeric, humanized such as CDR-grafted, human, single chain, and/or bispecific, as well as fragments, variants or derivatives thereof. Antibody fragments include those portions of the antibody which bind to an epitope on at least one B7-like polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies directed toward at least one B7-like polypeptide generally are produced in animals (*e.g.*, rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of a B7-like polypeptide and an adjuvant. It may be useful to conjugate a B7-like polypeptide to a carrier protein

that is immunogenic in the species to be immunized, such as keyhole limpet heocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-B7-like polypeptide antibody titer.

Monoclonal antibodies directed toward at least one B7-like polypeptide are produced using any method which provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler *et al.*, *Nature,* 256:495-497 (1975) and the human B-cell hybridoma method, Kozbor, *J. Immunol.,* 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York (1987)). Also provided by the invention are hybridoma cell lines which produce monoclonal antibodies reactive with at least one B7-like polypeptide.

Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired

biological activity. See, U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci.*, 81:6851-6855 (1985).

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. See U.S. Patent Nos. 5,585,089, and 5,693,762. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. Humanization can be performed, for example, using methods described in the art (Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeyen *et al.*, *Science*, 239:1534-1536 (1988)), by substituting at least a portion of a rodent complementarity-determining region (CDR) for the corresponding regions of a human antibody.

Also encompassed by the invention are human antibodies which bind at least one B7-like polypeptide. Using transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production, such antibodies are produced by immunization with a B7-like antigen (*i.e.*, having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, for example, Jakobovits *et al.*, *Proc. Natl. Acad. Sci.*, 90:2551-2555 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.*, 7:33 (1993). In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain

proteins into the genome thereof. Partially modified animals, that is those having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human (rather than *e.g.*, murine) amino acid sequences, including variable regions which are immunospecific for these antigens. See PCT Application NOs. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT Application NOs. PCT/US91/245 and PCT/GB89/01207, and in EP 546073B1 and EP 546073A1. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In an alternative embodiment, human antibodies can be produced from phage-display libraries (Hoogenboom *et al.*, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT Application No. PCT/US98/17364, which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk- receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures

described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (*e.g.*, human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-B7-like antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Sola, *Monoclonal Antibodies: A Manual of Techniques*, pp. 147-158, CRC Press, Inc. (1987)) for the detection and quantitation of B7-like polypeptides. The antibodies will bind B7-like polypeptides with an affinity which is appropriate for the assay method being employed.

For diagnostic applications, in certain embodiments, anti-B7-like antibodies may be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β-galactosidase, or horseradish peroxidase (Bayer *et al.*, *Meth. Enz.*, 184:138-163 (1990)).

Competitive binding assays rely on the ability of a labeled standard (*e.g.*, a B7-like polypeptide, or an immunologically reactive portion thereof) to compete with the test sample analyte (a B7-like polypeptide) for binding with a limited amount of anti-B7-like

antibody. The amount of B7-like polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex. See, *e.g.*, U.S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assays). For example, one type of sandwich assay is an enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The selective binding agents, including anti-B7-like antibodies, also are useful for *in vivo* imaging. An antibody labeled with a detectable moiety may be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host is assayed. The antibody may be labeled with any moiety that is detectable in an

animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

Selective binding agents of the invention, including antibodies, may be used as therapeutics. These therapeutic agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of at least one B7-like polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to at least one B7-like polypeptide and which are capable of inhibiting or eliminating the functional activity of the mature form of at least one B7-like polypeptide *in vivo* or *in vitro*. In preferred embodiments, the selective binding agent, *e.g.*, an antagonist antibody, will inhibit the functional activity of the mature form of at least one B7-like polypeptide by at least about 50%, and preferably by at least about 80%. In another embodiment, the selective binding agent may be an antibody that is capable of interacting with a B7-like binding partner (a ligand or receptor) thereby inhibiting or eliminating B7-like activity *in vitro* or *in vivo*. Selective binding agents, including agonist and antagonist anti-B7-like antibodies, are identified by screening assays which are well known in the art.

The invention also relates to a kit comprising B7-like selective binding agents (such as antibodies) and other reagents useful for detecting B7-like polypeptide levels in biological samples. Such reagents may include, a detectable label, blocking serum, positive and negative control samples, and detection reagents.

The B7-like polypeptides of the present invention can be used to clone B7-like receptors, using an expression cloning strategy. Radiolabeled (125-Iodine) B7-like polypeptide or affinity/activity-tagged B7-like polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or cell line or tissue that expresses B7-like receptor(s). RNA isolated from such cells or tissues can be converted to cDNA, cloned into a mammalian expression vector, and transfected into mammalian cells (such as COS or 293 cells) to create an expression library. A radiolabeled or tagged B7-like polypeptide can then be used as an affinity ligand to identify and isolate from this library the subset of cells which express the B7-like receptor(s) on their surface. DNA can then be isolated from these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing B7-like receptor(s) is many-fold higher than in the original library. This enrichment process can be repeated iteratively until a single recombinant clone containing a B7-like receptor is isolated. Isolation of the B7-like receptor(s) is useful for identifying or developing novel agonists and antagonists of the B7-like polypeptide signaling pathway. Such agonists and antagonists include soluble B7-like receptor(s), anti-B7-like receptor antibodies, small molecules, or antisense oligonucleotides, and they may be used for treating, preventing, or diagnosing one or more disease or disorders, including those described herein.

Assaying for other modulators of B7-like polypeptide activity

In some situations, it may be desirable to identify molecules that are modulators, *i.e.*, agonists or antagonists, of the activity of "the mature form of at least one B7-like polypeptide. Natural or synthetic molecules that modulate at least one B7-like polypeptide may be identified using one or more screening assays, such as those described herein. Such molecules may be administered either in an *ex vivo* manner, or in an *in vivo* manner by injection, or by oral delivery, implantation device, or the like.

"Test molecule(s)" refers to the molecule(s) that is/are under evaluation for the ability to modulate (*i.e.*, increase or decrease) the activity of the mature form of at least one B7-like polypeptide. Most commonly, a test molecule will interact directly with the mature form of at least one B7-like polypeptide. However, it is also contemplated that a test molecule may also modulate B7-like polypeptide activity indirectly, such as by affecting B7-like gene expression, or by binding to a B7-like binding partner (*e.g.*, receptor or ligand). In one embodiment, a test molecule will bind to at least one B7-like polypeptide with an affinity constant of at least about 10^{-6} M, preferably about 10^{-8} M, more preferably about 10^{-9} M, and even more preferably about 10^{-10} M.

Methods for identifying compounds which interact with at least one B7-like polypeptide are encompassed by the present invention. In certain embodiments, a B7-like polypeptide is incubated with a test molecule

under conditions which permit the interaction of the test molecule with a B7-like polypeptide, and the extent of the interaction can be measured. The test molecule(s) can be screened in a substantially purified form or in a crude mixture.

In certain embodiments, a B7-like polypeptide agonist or antagonist may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule which interacts with at least one B7-like polypeptide to regulate its activity. Molecules which regulate B7-like polypeptide expression include nucleic acids which are complementary to nucleic acids encoding a B7-like polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of at least one B7-like polypeptide, and which act as anti-sense regulators of expression.

Once a set of test molecules has been identified as interacting with at least one B7-like polypeptide, the molecules may be further evaluated for their ability to increase or decrease B7-like polypeptide activity. The measurement of the interaction of test molecules with at least one B7-like polypeptide may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays and immunoassays. In general, test molecules are incubated with a B7-like polypeptide for a specified period of time, and B7-like polypeptide activity is determined by one or more assays for measuring biological activity.

The interaction of test molecules with at least one B7-like polypeptide may also be assayed directly

using polyclonal or monoclonal antibodies in an immunoassay. Alternatively, modified forms of at least one B7-like polypeptide containing epitope tags as described herein may be used in immunoassays.

In the event that B7-like polypeptides display biological activity through an interaction with a binding partner (*e.g.*, a receptor or a ligand), a variety of *in vitro* assays may be used to measure the binding of such B7-like polypeptides to the corresponding binding partner (such as a selective binding agent, receptor or ligand). These assays may be used to screen test molecules for their ability to increase or decrease the rate and/or the extent of binding of a B7-like polypeptide to its binding partner. In one assay, a B7-like polypeptide is immobilized in the wells of a microtiter plate. Radiolabeled B7-like binding partner (for example, iodinated B7-like binding partner) and the test molecule(s) can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can be washed and counted, using a scintillation counter, for radioactivity to determine the extent to which the binding partner bound to the B7-like polypeptide. Typically, the molecules will be tested over a range of concentrations, and a series of control wells lacking one or more elements of the test assays can be used for accuracy in the evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins, *i.e.*, immobilizing a B7-like binding partner to the microtiter plate wells, incubating with the test molecule and radiolabeled B7-like polypeptide, and

determining the extent of B7-like polypeptide binding. See, for example, Chapter 18, *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, New York, NY (1995).

As an alternative to radiolabelling, a B7-like polypeptide or its binding partner may be conjugated to biotin and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), that can be detected colorometrically, or by fluorescent tagging of streptavidin. An antibody directed to a B7-like polypeptide or to a B7-like binding partner and conjugated to biotin may also be used and can be detected after incubation with enzyme-linked streptavidin linked to AP or HRP.

A B7-like polypeptide or a B7-like binding partner can also be immobilized by attachment to agarose beads, acrylic beads or other types of such inert solid phase substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound. After incubation, the beads can be precipitated by centrifugation, and the amount of binding between a B7-like polypeptide and its binding partner can be assessed using the methods described herein. Alternatively, the substrate-protein complex can be immobilized in a column, and the test molecule and complementary protein are passed through the column. The formation of a complex between a B7-like polypeptide and its binding partner can then be assessed using any of the techniques set forth herein, *i.e.*, radiolabelling, antibody binding, or the like.

Another *in vitro* assay that is useful for identifying a test molecule which increases or decreases the formation of a complex between a B7-like polypeptide and a B7-like binding partner is a surface plasmon resonance detector system such as the BIAcore assay system (Pharmacia, Piscataway, NJ). The BIAcore system may be carried out using the manufacturer's protocol. This assay essentially involves the covalent binding of either a B7-like polypeptide or a B7-like binding partner to a dextran-coated sensor chip which is located in a detector. The test compound and the other complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass which is physically associated with the dextran-coated side of the sensor chip; the change in molecular mass can be measured by the detector system.

In some cases, it may be desirable to evaluate two or more test compounds together for their ability to increase or decrease the formation of a complex between a B7-like polypeptide and a B7-like binding partner. In these cases, the assays set forth herein can be readily modified by adding such additional test compound(s) either simultaneous with, or subsequent to, the first test compound. The remainder of the steps in the assay are as set forth herein.

In vitro assays such as those described herein may be used advantageously to screen large numbers of compounds for effects on complex formation by a B7-like polypeptide and B7-like binding partner. The assays

may be automated to screen compounds generated in phage display, synthetic peptide, and chemical synthesis libraries.

Compounds which increase or decrease the formation of a complex between a B7-like polypeptide and a B7-like binding partner may also be screened in cell culture using cells and cell lines expressing either a B7-like polypeptide or a B7-like binding partner. Cells and cell lines may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of a B7-like polypeptide to cells expressing B7-like binding partner at the surface is evaluated in the presence or absence of test molecules, and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to a B7-like binding partner. Cell culture assays can be used advantageously to further evaluate compounds that score positive in protein binding assays described herein.

Cell cultures can also be used to screen the impact of a drug candidate. For example, drug candidates may decrease or increase the expression of a B7-like gene. In certain embodiments, the amount of B7-like polypeptide that is produced may be measured after exposure of the cell culture to the drug candidate. In certain embodiments, one may detect the actual impact of the drug candidate on the cell culture. For example, the overexpression of a particular gene may have a particular impact on the cell culture. In such cases, one may test a drug candidate's ability to increase or decrease the expression of the gene or its ability to prevent or

inhibit a particular impact on the cell culture. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product in a cell culture.

Internalizing Proteins

The *tat* protein sequence (from HIV) can be used to internalize proteins into a cell. See *e.g.*, Falwell *et al.*, *Proc. Natl. Acad. Sci.*, 91:664-668 (1994). For example, an 11 amino acid sequence (YGRKKRRQRRR) of the HIV tat protein (termed the "protein transduction domain", or TAT PDT) has been described as mediating delivery across the cytoplasmic membrane and the nuclear membrane of a cell. *See* Schwarze *et al.*, *Science*, 285:1569-1572 (1999); and Nagahara *et al.*, *Nature Medicine*, 4:1449-1452 (1998). In these procedures, FITC-constructs (FITC-GGGGYGRKKRRQRRR) are prepared which bind to cells as observed by fluorescence-activated cell sorting (FACS) analysis, and these constructs penetrate tissues after i.p. adminstration. Next, tat-bgal fusion proteins are constructed. Cells treated with this construct demonstrated b-gal activity. Following injection, a number of tissues, including liver, kidney, lung, heart, and brain tissue have been found to demonstrate expression using these procedures. It is believed that these constructions underwent some degree of unfolding in order to enter the cell; as such, refolding may be required after entering the cell.

It will thus be appreciated that the tat protein

sequence may be used to internalize a desired protein or polypeptide into a cell. For example, using the *tat* protein sequence, a B7-like antagonist (such as an anti-B7-like selective binding agent, small molecule, soluble receptor, or antisense oligonucleotide) can be administered intracellularly to inhibit the activity of the mature form of at least one B7-like molecule. As used herein, the term "B7-like molecules" refers to both B7-like nucleic acid molecules and B7-like polypeptides as defined herein. Where desired, the B7-like proteins may also be internally administered to a cell using these procedures. See also, Strauss, E., "Introducing Proteins Into the Body's Cells", *Science*, 285:1466-1467 (1999).

Therapeutic Uses

Polypeptides of the invention, and agonists and antagonists thereof, may be used to regulate T-cell function. Accordingly, B7-like polypeptides may be used to treat, diagnose, ameliorate, or prevent acute or chronic diseases associated with T-cell function.

Agonists and antagonists include those molecules which regulate B7-like polypeptide activity and either increase or decrease at least one activity of the mature form of at least one B7-like polypeptide such as one activity associated with T-cell functions, for example, T-cell activation. Agonists or antagonists may be co-factors, such as a protein, peptide, carbohydrate, lipid or small molecular weight molecule, which interact with at least one B7-like polypeptide and thereby regulate polypeptide activity. Potential polypeptide agonists or antagonists include antibodies

that react with either soluble or membrane-bound forms of at least one B7-like polypeptide which comprise part or all of the extracellular domains of the said proteins. Molecules that regulate B7-like polypeptide expression typically include nucleic acids that can act as anti-sense regulators of expression.

The phenotype of transgenic mice expressing B7-like polypeptide corresponding to SEQ ID NO: 14 showed seminal vesicle hyperplasia. Accordingly, agonists and antagonists of B7-like polypeptide activity may be useful in the treatment of reproductive disorders and proliferative disorders.

Antibodies, soluble proteins comprising for example extracellular domains, and other regulators of B7-like polypeptide expression that result in prolonged or enhanced T-cell activation can be used to increase the immune response to tumors. B7-like polypeptides may play a role in the growth and maintenance of cancer cells based on overexpression causing seminal vesicle hyperplasia. Accordingly, agonists or antagonists to B7-like polypeptides may be useful for the diagnosis and/or treatment of cancer. Examples of such cancers include, but are not limited to, seminal vesicle cancer, lung cancer, brain cancer, breast cancer, cancers of the hematopoetic system, prostate cancer, ovarian cancer, and testicular cancer. Other cancers are encompassed within the scope of the invention. The B7-like polypeptide pathway can also be manipulated to regulate CTL response in a number of other clinical settings, including allograft transplantation, graft vs. host disease, and autoimmune diseases.

B7-like polypeptides may play a role in the inappropriate proliferation of cells based on overexpression causing seminal vesicle hyperplasia.

Accordingly, agonists or antagonists to B7-like polypeptides may be useful for the diagnosis and/or treatment of diseases where there is abnormal cell proliferation. Examples of such diseases include, but are not limited to, arteriosclerosis and vascular restenosis. Other diseases influenced by the inappropriate proliferation of cells are encompassed within the scope of the invention.

B7-like polypeptides may play a role in the reproductive system based on overexpression causing seminal vesicle hyperplasia. Accordingly, agonists or antagonists to B7-like polypeptides may be useful for the diagnosis and/or treatment of reproductive disorders. Examples of such diseases include, but are not limited to, infertility, miscarriage, preterm labor and delivery, and endometriosis. Other diseases of the reproductive system are encompassed within the scope of the invention.

B7-like polypeptides, and agonists and antagonists thereof, may be used in the treatment of autoimmune disease, graft survival, immune cell activation for inhibiting tumor cell growth, T-cell dependent B-cell mediated diseases, and cancer gene immunotherapy. In one embodiment, antagonists or inhibitors of B7-like polypeptide function may be beneficial to alleviate symptoms in diseases with chronic immune cell dysfunction. Autoimmune diseases, such as systemic lupus erythematosis, rheumatoid arthritis, immune thrombocytopenic purpura (ITP) and psoriasis may be treated with B7-like polypeptide antagonists or inhibitors. In addition, chronic inflammatory diseases, such as inflammatory bowel disease (Crohn's disease and ulcerative colitis), Grave's disease,

Hashimoto's thyroiditis and diabetes mellitus may also be treated with B7-like polypeptide inhibitors.

B7-like polypeptide antagonists may be used as immunosuppressive agents for bone marrow and organ transplantation and may be used to prolong graft survival. Such antagonists may provide significant advantages over existing treatment. Bone marrow and organ transplantation therapy must contend with T-cell mediated rejection of the foreign cells or tissue by the host. Present therapeutic regimens for inhibiting T-cell mediated rejection involve treatment with the drugs cyclosporine or FK506. While drugs are effective, patients suffer from serious side effects, including hepatotoxicity, nephrotoxicity and neurotoxicity. The target for the cyclosporin/FK506 class of therapeutics is calcineurin, a phosphatase with ubiquitous expression. Inhibitors of B7-like polypeptides or proteins may lack the severe side effects observed with use of the present immunotherapeutic agents.

Antagonists of B7-like polypeptides or proteins may be used as immunosuppressive agents for autoimmune disorders, such as rheumatoid arthritis, psoriasis, multiple sclerosis, diabetes, and systemic lupus erythematosus.

Antagonists of the B7-like polypeptides or proteins may also be used to alleviate toxic shock syndrome, inflammatory bowel disease, allosensitization due to blood transfusions, T-cell dependent B-cell mediated diseases, and the treatment of graft vs. host disease.

Gene therapy using B7-like polypeptide or protein genes of the invention may be used in cancer

immunotherapy. B7-like polypeptide genes introduced into cancer cells can transform them into antigen presenting cells that can be recognized by the T-cells of the immune system when introduced back into an animal. Recognition of the transfected tumor cells by the T-cells results in eradication of both tumors cells expressing, or not expressing, the B7-like polypeptide gene. This immunotherapy approach may be used for various leukemias, sarcomas, melanomas, adenocarcinomas, breast carcinomas, prostate tumors, lung carcinomas, colon carcinomas and other tumors. This invention encompasses using the B7-like polypeptide gene in a similar manner to enhance T-cell activation in response to variety of tumors.

For instance, many vaccines act by eliciting an effective and specific antibody response. Some vaccines, especially those against intestinal micro-organisms (e.g. Hepatitis A virus, and Salmonellas), elicit a short-lived antibody response. It is desirable to potentiate and prolong this response in order to increase the effectiveness of the vaccine. Therefore, soluble B7-like polypeptides or proteins may serve as a vaccine adjuvant.

Anti-viral responses may also be enhanced by activators or agonists of the B7-like protein pathway. The enhancement of cellular immune functions by B7-like polypeptide or protein/-Fc, may also be beneficial in eliminating virus-infected cells. In a complementary fashion, B7-like polypeptide or protein/-Fc may also have effects on humoral immune functions that may enhance antibody mediated responses and that may function to help clear free-virus from the body.

Conversely, there are a number of clinical conditions that would be ameliorated by the inhibition

of antibody production. Hypersensitivity is a normally beneficial immune response that is exaggerated or inappropriate, and leads to inflammatory reactions and tissue damage. Hypersensitivity reactions which are antibody-mediated may be particularly susceptible to antagonism by inhibitors of B7-like polypeptide or protein activity. Allergies, hay fever, asthma and acute edema cause type-I hypersensitivity reactions, and these reactions may be suppressed by protein, antibody or small molecule inhibitors of B7-like polypeptide or protein activity.

Diseases that cause antibody-mediated hypersensitivity reactions, including systemic lupus erythematosis, arthritis (rheumatoid arthritis, reactive arthritis, psoriatic arthritis), nephropathies (glomerulo-nephritis, membranous, mesangiocapillary, focal segmental, focal necrotizing, crescentic, proliferative tubulopathies), skin disorders (pemphigus and pemphigoid, erythema nodosum), endocrinopathies (Grave's disease, Hashimoto's thyroiditis and diabetes mellitus), various pneumopathies (especially extrinsic alveolitis), various vasculopathies, coeliac disease, with aberrant production of IgA, many anemias and thrombocytopenias, Guillain-Barre syndrome, and myasthenia gravis may be treated with B7-like polypeptide or protein antagonists.

In addition, lymphoproliferative disorders, such as multiple myeloma, Waldenstrom's macroglobulinemia and crioglobulinemias may be inhibited by protein, antibody or small molecule antagonists of B7-like polypeptides or proteins.

Finally, graft versus host disease, an "artificial" immune disorder, may benefit from the

inhibition of antibody production by B7-like polypeptide or protein antagonists.

Other diseases associated with undesirable levels of one or more of the receptors of the present B7-like protein, and/or the present B7-like protein itself, are encompassed within the scope of the invention. Undesirable levels include excessive and/or sub-normal levels of the ligand of the present B7-like protein, and/or the B7-like protein described herein.

B7-like polypeptides, proteins, agonists and antagonists may be used in combination with cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

B7-like Compositions and Administration

Therapeutic compositions are within the scope of the present invention. Such B7-like pharmaceutical compositions may comprise a therapeutically effective amount of a B7-like polypeptide or a B7-like nucleic acid molecule in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Pharmaceutical compositions may comprise a therapeutically effective amount of one or more B7-like selective binding agents in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration.

Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine), antimicrobials, antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin), fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrins), proteins (such as serum albumin, gelatin or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide), solvents (such as glycerin, propylene glycol or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol),

suspending agents, surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal), stability enhancing agents (sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride), mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. (See *Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company (1990)).

The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See for example, *Remington's Pharmaceutical Sciences, supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the B7-like molecule.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present

invention, B7-like polypeptide compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized cake or an aqueous solution. Further, the B7-like polypeptide product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The B7-like pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired B7-like molecule in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a B7-like molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid,

polyglycolic acid), or beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered as a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In one embodiment, a pharmaceutical composition may be formulated for inhalation. For example, a B7-like molecule may be formulated as a dry powder for inhalation. B7-like polypeptide or B7-like nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT application no. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, B7-like molecules which are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the B7-like molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be

employed. Another pharmaceutical composition may involve an effective quantity of B7-like molecules in a mixture with non-toxic excipients which are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc. Additional B7-like pharmaceutical compositions will be evident to those skilled in the art, including formulations involving B7-like polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 which describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919 and EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15:167-277 (1981) and Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (Langer *et al.*, *supra*)

or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art. See *e.g.*, Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

The B7-like pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using these methods may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (*e.g.*, lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing

single and multi-chambered pre-filled syringes (*e.g.*, liquid syringes and lyosyringes).

An effective amount of a B7-like pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the B7-like molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 μg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 μg/kg up to about 100 mg/kg; or 1 μg/kg up to about 100 mg/kg; or 5 μg/kg up to about 100 mg/kg.

The frequency of dosing will depend upon the pharmacokinetic parameters of the B7-like molecule in the formulation used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by

them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, *e.g.* oral, injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, or intralesional routes, or by sustained release systems or implantation device. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or other appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed release bolus, or continuous administration.

In some cases, it may be desirable to use B7-like pharmaceutical compositions in an *ex vivo* manner. In such instances, cells, tissues, or organs that have been removed from the patient are exposed to B7-like pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, a B7-like polypeptide can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the

polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Additional embodiments of the present invention relate to cells and methods (*e.g.*, homologous recombination and/or other recombinant production methods) for both the *in vitro* production of therapeutic polypeptides and for the production and delivery of therapeutic polypeptides by gene therapy or cell therapy. Homologous and other recombination methods may be used to modify a cell that contains a normally transcriptionally silent B7-like gene, or an under-expressed gene, and thereby produce a cell which expresses therapeutically efficacious amounts of B7-like polypeptides.

Homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes (Kucherlapati, *Prog. in Nucl. Acid Res. & Mol. Biol.*, 36:301, 1989). The basic technique was developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas *et al.*, *Cell*, 44:419-428, 1986; Thomas and Capecchi, *Cell*, 51:503-512, 1987 and Doetschman *et al.*, *Proc. Natl. Acad.*

Sci., 85:8583-8587, 1988) or to correct specific mutations within defective genes (Doetschman *et al.*, *Nature*, 330:576-578, 1987). Exemplary homologous recombination techniques are described in U.S. Patent No. 5,272,071 (EP 9193051, EP Publication No. 505500; PCT/US90/07642, International Publication No. WO 91/09955).

Through homologous recombination, the DNA sequence to be inserted into the genome can be directed to a specific region of the gene of interest by attaching it to targeting DNA. The targeting DNA is a nucleotide sequence that is complementary (homologous) to a region of the genomic DNA. Small pieces of targeting DNA that are complementary to a specific region of the genome are put in contact with the parental strand during the DNA replication process. It is a general property of DNA that has been inserted into a cell to hybridize, and therefore, recombine with other pieces of endogenous DNA through shared homologous regions. If this complementary strand is attached to an oligonucleotide that contains a mutation or a different sequence or an additional nucleotide, it too is incorporated into the newly synthesized strand as a result of the recombination. As a result of the proofreading function, it is possible for the new sequence of DNA to serve as the template. Thus, the transferred DNA is incorporated into the genome.

Attached to these pieces of targeting DNA are regions of DNA which may interact with or control the expression of a B7-like polypeptide, *e.g.*, flanking sequences. For example, a promoter/enhancer element, a suppresser, or an exogenous transcription modulatory

element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired B7-like polypeptide. The control element controls a portion of the DNA present in the host cell genome. Thus, the expression of the desired B7-like polypeptide may be achieved not by transfection of DNA that encodes the B7-like gene itself, but rather by the use of targeting DNA (containing regions of homology with the endogenous gene of interest) coupled with DNA regulatory segments that provide the endogenous gene sequence with recognizable signals for transcription of a B7-like polypeptide.

In an exemplary method, the expression of a desired targeted gene in a cell (*i.e.*, a desired endogenous cellular gene) is altered via homologous recombination into the cellular genome at a preselected site, by the introduction of DNA which includes at least a regulatory sequence, an exon and a splice donor site. These components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit (in which the regulatory sequence, the exon and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of these components into the chromosomal DNA, the expression of the desired endogenous gene is altered.

Altered gene expression, as described herein, encompasses activating (or causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a

gene which is not expressed at physiologically significant levels in the cell as obtained. The embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regulation or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

One method by which homologous recombination can be used to increase, or cause, B7-like polypeptide production from a cell's endogenous B7-like gene involves first using homologous recombination to place a recombination sequence from a site-specific recombination system (*e.g.*, Cre/loxP, FLP/FRT) (Sauer, *Current Opinion In Biotechnology*, 5:521-527, 1994 and Sauer, *Methods In Enzymology*, 225:890-900, 1993) upstream (that is, 5' to) of the cell's endogenous genomic B7-like polypeptide coding region. A plasmid containing a recombination site homologous to the site that was placed just upstream of the genomic B7-like polypeptide coding region is introduced into the modified cell line along with the appropriate recombinase enzyme. This recombinase causes the plasmid to integrate, via the plasmid's recombination site, into the recombination site located just upstream of the genomic B7-like polypeptide coding region in the cell line (Baubonis and Sauer, *Nucleic Acids Res.*, 21:2025-2029, 1993 and O'Gorman *et al.*, *Science*, 251:1351-1355, 1991). Any flanking sequences known to increase transcription (*e.g.*, enhancer/promoter, intron, translational enhancer), if properly positioned in this plasmid, would integrate in such a manner as to

create a new or modified transcriptional unit resulting in *de novo* or increased B7-like polypeptide production from the cell's endogenous B7-like gene.

A further method to use the cell line in which the site specific recombination sequence had been placed just upstream of the cell's endogenous genomic B7-like polypeptide coding region is to use homologous recombination to introduce a second recombination site elsewhere in the cell line's genome. The appropriate recombinase enzyme is then introduced into the two-recombination-site cell line, causing a recombination event (deletion, inversion, translocation) (Sauer, *Current Opinion In Biotechnology*, *supra*, 1994; Sauer, *Methods In Enzymology*, *supra*, 1993) that would create a new or modified transcriptional unit resulting in *de novo* or increased B7-like polypeptide production from the cell's endogenous B7-like gene.

An additional approach for increasing, or causing, the expression of B7-like polypeptide from a cell's endogenous B7-like gene involves increasing, or causing, the expression of a gene or genes (*e.g.*, transcription factors) and/or decreasing the expression of a gene or genes (*e.g.*, transcriptional repressors) in a manner which results in *de novo* or increased B7-like polypeptide production from the cell's endogenous B7-like gene. This method includes the introduction of a non-naturally occurring polypeptide (*e.g.*, a polypeptide comprising a site specific DNA binding domain fused to a transcriptional factor domain) into the cell such that *de novo* or increased B7-like polypeptide production from the cell's endogenous B7-like gene results.

The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences; (b) a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. The targeting sequence in the DNA construct directs the integration of elements (a)-(d) into a target gene in a cell such that the elements (b)-(d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)-(f) such that the elements of (b)-(f) are operatively linked to the endogenous gene. The targeting sequence is homologous to the preselected site in the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3′ of the regulatory sequence and the splice-donor site is 3′ of the exon.

If the sequence of a particular gene is known, such as the nucleic acid sequence of B7-like polypeptide presented herein, a piece of DNA that is complementary to a selected region of the gene can be synthesized or otherwise obtained, such as by appropriate restriction of the native DNA at specific recognition sites bounding the region of interest. This piece serves as a targeting sequence(s) upon insertion into the cell and will hybridize to its homologous region within the genome. If this hybridization occurs during DNA replication, this piece

of DNA, and any additional sequence attached thereto, will act as an Okazaki fragment and will be incorporated into the newly synthesized daughter strand of DNA. The present invention, therefore, includes nucleotides encoding a B7-like polypeptide, which nucleotides may be used as targeting sequences.

B7-like polypeptide cell therapy, *e.g.*, the implantation of cells producing B7-like polypeptides, is also contemplated. This embodiment involves implanting cells capable of synthesizing and secreting a biologically active form of B7-like polypeptide. Such B7-like polypeptide-producing cells can be cells that are natural producers of B7-like polypeptides or may be recombinant cells whose ability to produce B7-like polypeptides has been augmented by transformation with a gene encoding the desired B7-like polypeptide or with a gene augmenting the expression of B7-like polypeptide. Such a modification may be accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered a B7-like polypeptide, as may occur with the administration of a polypeptide of a foreign species, it is preferred that the natural cells producing B7-like polypeptide be of human origin and produce human B7-like polypeptide. Likewise, it is preferred that the recombinant cells producing B7-like polypeptide be transformed with an expression vector containing a gene encoding a human B7-like polypeptide.

Implanted cells may be encapsulated to avoid the infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in

biocompatible, semipermeable polymeric enclosures or membranes that allow the release of B7-like polypeptide, but that prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce B7-like polypeptides *ex vivo*, may be implanted directly into the patient without such encapsulation.

Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be routinely accomplished. For example, Baetge *et al.* (WO95/05452; PCT/US94/09299) describe membrane capsules containing genetically engineered cells for the effective delivery of biologically active molecules. The capsules are biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively linked to promoters that are not subject to down regulation *in vivo* upon implantation into a mammalian host. The devices provide for the delivery of the molecules from living cells to specific sites within a recipient. In addition, see U.S. Patent Nos. 4,892,538, 5,011,472, and 5,106,627. A system for encapsulating living cells is described in PCT Application no. PCT/US91/00157 (Aebischer *et al*). See also, PCT Application no. PCT/US91/00155 Aebischer *et al.*, Winn *et al.*, *Exper. Neurol.*, 113:322-329 (1991), Aebischer *et al.*, *Exper. Neurol.*, 111:269-275 (1991); and Tresco *et al.*, ASAIO, 38:17-23 (1992).

In vivo and *in vitro* gene therapy delivery of B7-like polypeptides is also envisioned. One example of a gene therapy technique is to use the B7-like gene (either genomic DNA, cDNA, and/or synthetic DNA) encoding a B7-like polypeptide which may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct". The promoter may be homologous or heterologous to the endogenous B7-like gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include, DNA molecules designed for site-specific integration (*e.g.*, endogenous sequences useful for homologous recombination), tissue-specific promoter, enhancer(s) or silencer(s), DNA molecules capable of providing a selective advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (as, for example, for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as factors to enable vector manufacture.

A gene therapy DNA construct can then be introduced into cells (either *ex vivo* or *in vivo*) using viral or non-viral vectors. One means for introducing the gene therapy DNA construct is by means of viral vectors as described herein. Certain vectors, such as retroviral vectors, will deliver the DNA construct to the chromosomal DNA of the cells, and the gene can integrate into the chromosomal DNA. Other vectors will function as episomes, and the gene therapy DNA construct will remain in the cytoplasm.

In yet other embodiments, regulatory elements can be included for the controlled expression of the B7-like gene in the target cell. Such elements are turned on in response to an appropriate effector. In this way, a therapeutic polypeptide can be expressed when desired. One conventional control means involves the use of small molecule dimerizers or rapalogs (as described in WO9641865 (PCT/US96/099486); WO9731898 (PCT/US97/03137) and WO9731899 (PCT/US95/03157)) used to dimerize chimeric proteins which contain a small molecule-binding domain and a domain capable of initiating biological process, such as a DNA-binding protein or transcriptional activation protein. The dimerization of the proteins can be used to initiate transcription of the transgene.

An alternative regulation technology uses a method of storing proteins expressed from the gene of interest inside the cell as an aggregate or cluster. The gene of interest is expressed as a fusion protein that includes a conditional aggregation domain which results in the retention of the aggregated protein in the endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can be released, however, by administering a drug (*e.g.*, small molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the aggregates or clusters so that the proteins may be secreted from the cell. See, *Science* 287:816-817, and 826-830 (2000).

Other suitable control means or gene switches include, but are not limited to, the following systems. Mifepristone (RU486) is used as a progesterone

antagonist. The binding of a modified progesterone receptor ligand-binding domain to the progesterone antagonist activates transcription by forming a dimer of two transcription factors which then pass into the nucleus to bind DNA. The ligand binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further described in U.S. 5,364,791; WO9640911, and WO9710337.

Yet another control system uses ecdysone (a fruit fly steroid hormone) which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation domain/DNA-binding domain/ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. 5,514,578; WO9738117; WO9637609; and WO9303162.

Another control means uses a positive tetracycline-controllable transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated tet R-4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, *i.e.*, it binds to a tet operator in the presence of tetracycline) linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Nos. 5,464,758; 5,650,298 and 5,654,168.

Additional expression control systems and nucleic acid constructs are described in U.S. Patent Nos.

5,741,679 and 5,834,186, to Innovir Laboratories Inc.

In vivo gene therapy may be accomplished by introducing the gene encoding a B7-like polypeptide into cells via local injection of a B7-like nucleic acid molecule or by other appropriate viral or non-viral delivery vectors. See Hefti, *Neurobiology*, 25:1418-1435 (1994). For example, a nucleic acid molecule encoding a B7-like polypeptide may be contained in an adeno-associated virus (AAV) vector for delivery to the targeted cells (*e.g.*, Johnson, International Publication No. WO95/34670; International Application No. PCT/US95/07178). The recombinant AAV genome typically contains AAV inverted terminal repeats flanking a DNA sequence encoding a B7-like polypeptide operably linked to functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not limited to, retrovirus, adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 5,399,346 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells which have been treated *in vitro* to insert a DNA segment encoding a therapeutic protein. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 5,631,236 involving adenoviral vectors; U.S. Patent No. 5,672,510 involving retroviral vectors; and U.S.

5,635,399 involving retroviral vectors expressing cytokines.

Nonviral delivery methods include, but are not limited to, liposome-mediated transfer, naked DNA delivery (direct injection), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (*e.g.*, gene gun). Gene therapy materials and methods may also include the use of inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to identify transformed cells, negative selection systems and expression control systems (safety measures), cell-specific binding agents (for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent No. 4,970,154 involving electroporation techniques; WO96/40958 involving nuclear ligands; U.S. Patent No. 5,679,559 describing a lipoprotein-containing system for gene delivery; U.S. Patent No. 5,676,954 involving liposome carriers; U.S. Patent No. 5,593,875 concerning methods for calcium phosphate transfection; and U.S. Patent No. 4,945,050 wherein biologically active particles are propelled at cells at a speed whereby the particles penetrate the surface of the cells and become incorporated into the interior of the cells.

It is also contemplated that B7-like gene therapy

or cell therapy can further include the delivery of one or more additional polypeptide(s) in the same or a different cell(s). For example, the host cell may be modified to express and release both B7-like polypeptide and cytokines, growth factors and anti-inflammatories. Alternatively, the B7-like polypeptide and cytokines, growth factors and anti-inflammatories, or B7-like polypeptide and cytokines, growth factors and anti-inflammatories, may be expressed in and released from separate cells.

Such cells may be separately introduced into the patient, or the cells may be contained in a single implantable device, such as the encapsulating membrane described above, or the cells may be separately modified by means of viral vectors.

A means to increase endogenous B7-like polypeptide expression in a cell via gene therapy is to insert one or more enhancer elements into the B7-like polypeptide promoter, where the enhancer element(s) can serve to increase transcriptional activity of the B7-like gene. The enhancer element(s) used will be selected based on the tissue in which one desires to activate the gene(s); enhancer elements known to confer promoter activation in that tissue will be selected. For example, if a gene encoding a B7-like polypeptide is to be "turned on" in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing the B7-like polypeptide promoter (and optionally, inserted into a vector and/or 5′ and/or 3′ flanking sequence(s), etc.) using standard cloning techniques. This construct,

known as a "homologous recombination construct", can then be introduced into the desired cells either *ex vivo* or *in vivo*.

Gene therapy also can be used to decrease B7-like polypeptide expression by modifying the nucleotide sequence of the endogenous promoter(s). Such modification is typically accomplished via homologous recombination methods. For example, a DNA molecule containing all or a portion of the promoter of the B7-like gene(s) selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. For example the TATA box and/or the binding site of a transcriptional activator of the promoter may be deleted using standard molecular biology techniques; such deletion can inhibit promoter activity thereby repressing the transcription of the corresponding B7-like gene. The deletion of the TATA box or the transcription activator binding site in the promoter may be accomplished by generating a DNA construct comprising all or the relevant portion of the B7-like polypeptide promoter(s) (from the same or a related species as the B7-like gene(s) to be regulated) in which one or more of the TATA box and/or transcriptional activator binding site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. The construct will typically contain at least about 500 bases of DNA that correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified. The construct may be introduced into the appropriate cells (either *ex vivo* or *in vivo*)

either directly or via a viral vector as described herein. Typically, the integration of the construct into the genomic DNA of the cells will be via homologous recombination, where the 5′ and 3′ DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

Additional Uses of B7-like Nucleic Acids and Polypeptides

Nucleic acid molecules of the present invention (including those that do not themselves encode biologically active polypeptides) may be used to map the locations of the B7-like gene and related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and *in situ* hybridization.

The B7-like polypeptides may be used (simultaneously or sequentially) in combination with one or more cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

Other methods may also be employed where it is desirable to inhibit the activity of one or more B7-like polypeptides. Such inhibition may be effected by nucleic acid molecules which are complementary to and hybridize to expression control sequences (triple helix formation) or to B7-like mRNA. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of the selected B7-like gene(s) can be introduced into the cell. Anti-sense probes may be designed by available techniques

using the sequence of B7-like polypeptide disclosed herein. Typically, each such antisense molecule will be complementary to the start site (5' end) of each selected B7-like gene. When the antisense molecule then hybridizes to the corresponding B7-like mRNA, translation of this mRNA is prevented or reduced. Antisense inhibitors provide information relating to the decrease or absence of a B7-like polypeptide in a cell or organism.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more B7-like polypeptides. In this situation, the DNA encoding a mutant polypeptide of each selected B7-like polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

In addition, a B7-like polypeptide, whether biologically active or not, may be used as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised. Selective binding agents that bind to a B7-like polypeptide (as described herein) may be used for *in vivo* and *in vitro* diagnostic purposes, including, but not limited to, use in labeled form to detect the presence of B7-like polypeptide in a body fluid or cell sample. The antibodies may also be used to prevent, treat, or diagnose a number of diseases and disorders, including those recited herein. The antibodies may bind to a B7-like polypeptide so as to diminish or block at least one activity characteristic of a B7-like

polypeptide, or may bind to a polypeptide to increase at least one activity characteristic of a B7-like polypeptide (including by increasing the pharmacokinetics of the B7-like polypeptide).

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

A-692

WHAT IS CLAIMED

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) the nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5 or 7;

(b) the nucleotide sequence as set forth in SEQ ID NOs: 9, 11 or 13;

(c) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) a nucleotide sequence encoding the polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of (a) or (b), wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14; and

(g) a nucleotide sequence complementary to any of (a)-(f).

2. An isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the polypeptide as set

forth in SEQ ID NOs: 2, 4, 6 or 8, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) a nucleotide sequence encoding a polypeptide that is at least about 70, 75, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the polypeptide as set forth in SEQ ID NOs: 10, 12 or 14, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(c) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5 or 7, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in SEQ ID NOs: 9, 11 or 13, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) a nucleotide sequence of SEQ ID NOs: 1, 3, 5 or 7, or (a) or (b), above, encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) a nucleotide sequence of SEQ ID NOs: 9, 11 or 13, or (a) or (b), above, encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10,

12 or 14;

g) a nucleotide sequence encoding a polypeptide that has a substitution and/or deletion of 1 to 100 amino acid residues as set forth in any of SEQ ID NOs: 1, 3, 5 or 7, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

h) a nucleotide sequence encoding a polypeptide that has a substitution and/or deletion of 1 to 100 amino acid residues as set forth in any of SEQ ID NOs: 9, 11 or 13, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(i) a nucleotide sequence of SEQ ID NOs: 1, 3, 5 or 7, or (a), (c), (e) or (g), above, comprising a fragment of at least about 16 nucleotides;

(j) a nucleotide sequence of SEQ ID NOs: 9, 11 or 13, or (b), (d), (f) or (h), above, comprising a fragment of at least about 16 nucleotides;

(k) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a), (c), (e), (g) or (i) , above,, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(l) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (b), (d), (f), (h) or (j), above, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14; and

(m) a nucleotide sequence complementary to any of (a)-(l).

3. An isolated nucleic acid molecule comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(c) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) a nucleotide sequence encoding a polypeptide

as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(g) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(h) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(i) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one modification selected from at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(j) a nucleotide sequence encoding a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14 with at least one modification selected from at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(k) a nucleotide sequence of (a)-(j) comprising a fragment of at least about 16 nucleotides;

(l) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a), (c), (e), (g), (i) or (k), wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(m) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (b), (d), (f), (h), (j) or (k), wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;and

(n) a nucleotide sequence complementary to any of (a)-(m).

4. A vector comprising the nucleic acid molecule of Claims 1, 2, or 3.

5. A host cell comprising the vector of Claim 4.

6. The host cell of Claim 5 that is a eukaryotic cell.

7. The host cell of Claim 5 that is a prokaryotic cell.

8. A process of producing a B7-like polypeptide comprising culturing the host cell of Claim 5 under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

9. A polypeptide produced by the process of Claim 8.

10. The process of Claim 8, wherein the nucleic acid molecule comprises promoter DNA other than the promoter DNA for the native B7-like polypeptide operatively linked to the DNA encoding the B7-like polypeptide.

11. The isolated nucleic acid molecule according to Claim 2 wherein the percent identity is determined using a computer program selected from GAP, BLASTP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

12. A process for determining whether a compound inhibits B7-like polypeptide activity or production comprising exposing a cell according to Claims 5, 6, or 7 to the compound, and measuring B7-like polypeptide activity or production in said cell.

13. An isolated polypeptide comprising an amino acid sequence set forth in SEQ ID NOs: 2, 4, 6, or 8.

14. An isolated polypeptide comprising the amino acid sequence selected from:

a) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 2, and optionally further comprising an amino-terminal methionine;

(b) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 4, and optionally further comprising an amino-terminal methionine;

(c) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 6, and optionally further comprising an amino-terminal methionine;

(d) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 8, and optionally further comprising an amino-terminal methionine;

(e) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 10, and optionally further comprising an amino-terminal methionine;

(f) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 12, and optionally further comprising an amino-terminal methionine;

(g) an amino acid sequence comprising the mature form of the polypeptide of SEQ ID NO: 14, and optionally further comprising an amino-terminal methionine;

(h) an amino acid sequence for an ortholog of any one of SEQ ID NOs: 2, 4, 6 or 8, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(i) an amino acid sequence for an ortholog of any one of SEQ ID NOs: 10, 12 or 14, wherein the encoded polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(j) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence of SEQ ID NOs: 2, 4, 6 or 8, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4,

6 or 8;

(k) an amino acid sequence that is at least about 70, 80, 85, 90, 95, 96, 97, 98 or 99 percent identical to the amino acid sequence of SEQ ID NOs: 10, 12 or 14, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(l) a fragment of the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(m) a fragment of the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 comprising at least about 25 amino acid residues, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(n) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8, or at least one of (a), (c), (e), (f), (h), (i), (k) or (l), above, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8; and

(o) an amino acid sequence for an allelic variant or splice variant of either the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14, or at least one of (b), (d), (f), (h), (j), (l) or (m) wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14.

15. An isolated polypeptide comprising the amino

acid sequence selected from:

(a) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(b) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(c) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(d) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid insertion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(e) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(f) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 with at least one amino acid deletion, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(g) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8;

(h) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14 which has a C- and/or N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14;

(i) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 or 8, with at least one modification selected from at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 2, 4, 6 or 8; and

(j) the amino acid sequence as set forth in SEQ ID NOs: 10, 12 or 14, with at least one modification selected from at least one amino acid substitution, amino acid insertion, amino acid deletion, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the mature form of a polypeptide as set forth in SEQ ID NOs: 10, 12 or 14.

16. An isolated polypeptide encoded by a nucleic acid molecule of Claims 1, 2, or 3.

17. The isolated polypeptide according to Claim 14 wherein the percent identity is determined using a computer program selected from GAP, BLASTP, BLASTN,

FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

18. An antibody produced by immunizing an animal with a peptide comprising an amino acid sequence of SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

19. An antibody or fragment thereof that specifically binds at least one polypeptide of Claims 13, 14, or 15.

20. The antibody of Claim 19 that is a monoclonal antibody.

21. A hybridoma that produces a monoclonal antibody that binds to at least one peptide comprising an amino acid sequence selected from SEQ ID NOs: 2, 4, 6, 8, 10, 12 or 14.

22. A method of detecting or quantitating the amount of B7-like polypeptide using the anti-B7-like antibody or fragment of Claims 18, 19, or 20.

23. A selective binding agent or fragment thereof that specifically binds at least one polypeptide comprising an amino acid sequence selected from:

a) the amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 ,8, 10, 12 or 14; and

b) a fragment of the amino acid sequence set forth in at least one of SEQ ID NOs: 2, 4, 6 ,8, 10, 12 or 14; and

c) a naturally occurring variant of (a) or (b).

24. The selective binding agent of Claim 23 that is an antibody or fragment thereof.

25. The selective binding agent of Claim 23 that is a humanized antibody.

26. The selective binding agent of Claim 23 that is a human antibody or fragment thereof.

27. The selective binding agent of Claim 23 that is a polyclonal antibody or fragment thereof.

28. The selective binding agent Claim 23 that is a monoclonal antibody or fragment thereof.

29. The selective binding agent of Claim 23 that is a chimeric antibody or fragment thereof.

30. The selective binding agent of Claim 23 that is a CDR-grafted antibody or fragment thereof.

31. The selective binding agent of Claim 23 that is an antiidiotypic antibody or fragment thereof.

32. The selective binding agent of Claim 23 which is a variable region fragment.

33. The variable region fragment of Claim 32 which is a Fab or a Fab' fragment.

34. A selective binding agent or fragment thereof comprising at least one complementarity determining region with specificity for at least one polypeptide

comprising an amino acid sequence selected from SEQ ID NOs: 2, 4, 6 ,8, 10, 12 or 14.

35. The selective binding agent of Claim 23 which is bound to a detectable label.

36. The selective binding agent of Claim 23 which antagonizes B7-like polypeptide biological activity.

37. A method for treating, preventing, or ameliorating a disease, condition, or disorder comprising administering to a patient an effective amount of a selective binding agent according to Claim 23.

38. A selective binding agent produced by immunizing an animal with a polypeptide comprising an amino acid sequence selected SEQ ID NOs: 2, 4, 6 ,8, 10, 12 or 14.

39. A hybridoma that produces a selective binding agent capable of binding a polypeptide according to Claims 1, 2, or 3.

40. A composition comprising the polypeptide of Claims 13, 14, or 15 and a pharmaceutically acceptable formulation agent.

41. The composition of Claim 40 wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or anti-oxidant.

42. The composition of Claim 40 wherein the polypeptide comprises the mature form of an amino acid sequence as set forth in SEQ ID NOs: 2, 4, 6 ,8, 10, 12 or 14.

43. A polypeptide comprising a derivative of the polypeptide of Claims 13, 14, or 15.

44. The polypeptide of Claim 43 which is covalently modified with a water-soluble polymer.

45. The polypeptide of Claim 44 wherein the water-soluble polymer is selected from polyethylene glycol, monomethoxy-polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, and polyvinyl alcohol.

46. A composition comprising a nucleic acid molecule of Claims 1, 2, or 3 and a pharmaceutically acceptable formulation agent.

47. A composition of Claim 46 wherein said nucleic acid molecule is contained in a viral vector.

48. A viral vector comprising a nucleic acid molecule of Claims 1, 2, or 3.

49. A fusion polypeptide comprising the polypeptide of Claims 13, 14, or 15 fused to a heterologous amino acid sequence.

50. The fusion polypeptide of Claim 49 wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

51. A method for treating, preventing or ameliorating a medical condition comprising administering to a patient the polypeptide of Claims 13, 14, or 15 or the polypeptide encoded by the nucleic acid of Claims 1, 2, or 3.

52. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of Claims 13, 14, or 15 or the polypeptide encoded by the nucleic acid molecule of Claims 1, 2, or 3 in a sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

53. A device, comprising:

(a) a membrane suitable for implantation; and

(b) cells encapsulated within said membrane, wherein said cells secrete a protein of Claims 13, 14, or 15, and wherein said membrane is permeable to said protein and impermeable to materials detrimental to said cells.

54. A method of identifying a compound which binds to a polypeptide comprising:

(a) contacting the polypeptide of Claims 13, 14, or 15 with a compound; and

(b) determining the extent of binding of the polypeptide to the compound.

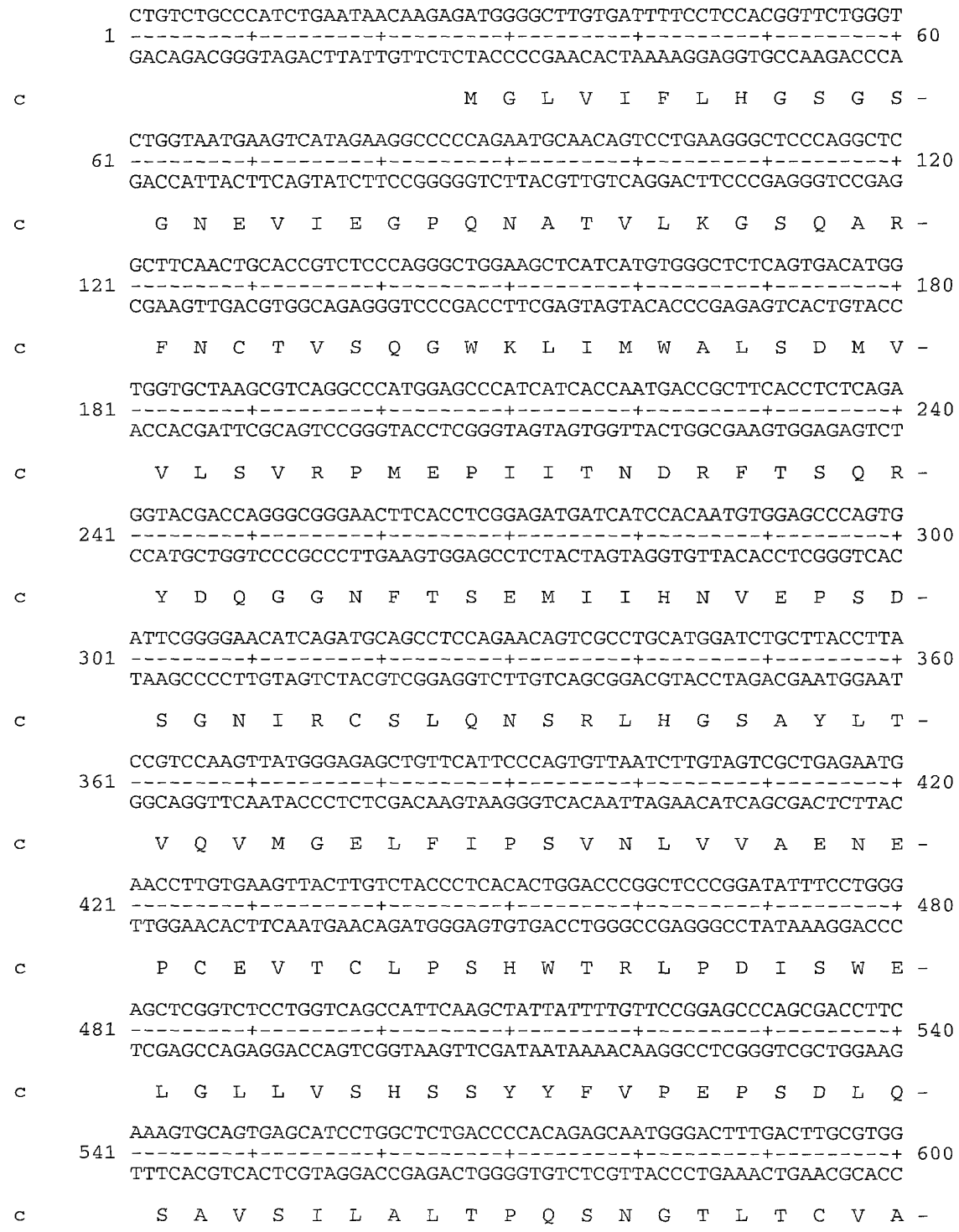
55. A method of modulating levels of a polypeptide in an animal comprising administering to the animal the nucleic acid molecule of Claims 1, 2, or 3.

56. A transgenic non-human mammal comprising the nucleic acid molecule of Claims 1, 2, or 3.

Abstract

Novel B7-like polypeptides and nucleic acid molecules encoding the same. The invention also provides vectors, host cells, selective binding agents, and methods for producing B7-like polypeptides. Also provided for are methods for the treatment, diagnosis, amelioration, or prevention of diseases with B7-like polypeptides.

FIGURE 1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61
120
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c G N E V I E G P Q N A T V L K G S Q A R -
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121
180
CGAAGTTGACGTGGCAGAGGGTCCCGACCTTCGAGTAGTACACCCGAGAGTCACTGTACC
c F N C T V S Q G W K L I M W A L S D M V -
TGGTGCTAAGCGTCAGGCCCATGGAGCCCATCATCACCAATGACCGCTTCACCTCTCAGA
181
240
ACCACGATTCGCAGTCCGGGTACCTCGGGTAGTAGTGGTTACTGGCGAAGTGGAGAGTCT
c V L S V R P M E P I I T N D R F T S Q R -
GGTACGACCAGGGCGGGAACTTCACCTCGGAGATGATCATCCACAATGTGGAGCCCAGTG
241
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ATTCGGGGAACATCAGATGCAGCCTCCAGAACAGTCGCCTGCATGGATCTGCTTACCTTA
301
360
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c S G N I R C S L Q N S R L H G S A Y L T -
CCGTCCAAGTTATGGGAGAGCTGTTCATTCCCAGTGTTAATCTTGTAGTCGCTGAGAATG
361
420
GGCAGGTTCAATACCCTCTCGACAAGTAAGGGTCACAATTAGAACATCAGCGACTCTTAC
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AACCTTGTGAAGTTACTTGTCTACCCTCACACTGGACCCGGCTCCCGGATATTTCCTGGG
421
480
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481
540
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c L G L L V S H S S Y Y F V P E P S D L Q -
AAAGTGCAGTGAGCATCCTGGCTCTGACCCCACAGAGCAATGGGACTTTGACTTGCGTGG
541
600
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c S A V S I L A L T P Q S N G T L T C V A -

FIGURE 1 (con't)

```
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    601 ---------+---------+---------+---------+---------+---------+ 660
        GATGGACCTTCTCGGACTTCCGGGCGTTCAGACGTTGACATTTAGAGTGACACTAAGCCA

c         T  W  K  S  L  K  A  R  K  S  A  T  V  N  L  T  V  I  R  C -

        GTCCCCAAGACACTGGAGGTGGTATTAATATTCCAGGTGTATTATCAAGTTTACCGAGTT
    661 ---------+---------+---------+---------+---------+---------+ 720
        CAGGGGTTCTGTGACCTCCACCATAATTATAAGGTCCACATAATAGTTCAAATGGCTCAA

c         P  Q  D  T  G  G  G  I  N  I  P  G  V  L  S  S  L  P  S  L -

        TAGGTTTTTCATTGCCTACTTGGGGCAAAGTTGGACTTGGACTAGCAGGCACCATGCTTC
    721 ---------+---------+---------+---------+---------+---------+ 780
        ATCCAAAAAGTAACGGATGAACCCCGTTTCAACCTGAACCTGATCGTCCGTGGTACGAAG

c         G  F  S  L  P  T  W  G  K  V  G  L  G  L  A  G  T  M  L  L -

        TGACGCCGACGTGTACTCTTACAATACGCTGCTGCTGCTGCCGCCGTCGTTGTTGTGGCT
    781 ---------+---------+---------+---------+---------+---------+ 840
        ACTGCGGCTGCACATGAGAATGTTATGCGACGACGACGACGGCGGCAGCAACAACACCGA

c         T  P  T  C  T  L  T  I  R  C  C  C  C  R  R  R  C  C  G  C -

        GCAACTGCTGCTGCCGTTGTTGTTTCTGCTGTAGAAGAAAAAGAGGATTTCGTATTCAAT
    841 ---------+---------+---------+---------+---------+---------+ 900
        CGTTGACGACGACGGCAACAACAAAGACGACATCTTCTTTTTCTCCTAAAGCATAAGTTA

c         N  C  C  C  R  C  C  F  C  C  R  R  K  R  G  F  R  I  Q  F -

        TTCAAAAGAAATCTGAAAAAGAGAAGACAAACAAAGAAACTGAGACAGAAAGTGGAAATG
    901 ---------+---------+---------+---------+---------+---------+ 960
        AAGTTTTCTTTAGACTTTTTCTCTTCTGTTTGTTTCTTTGACTCTGTCTTTCACCTTTAC

c         Q  K  K  S  E  K  E  K  T  N  K  E  T  E  T  E  S  G  N  E -

        AAAACTCCGGCTACAATTCAGATGAACAAAAGACCACAGACACCGCTTCTCTCCCTCCCA
    961 ---------+---------+---------+---------+---------+---------+ 1020
        TTTTGAGGCCGATGTTAAGTCTACTTGTTTTCTGGTGTCTGTGGCGAAGAGAGGGAGGGT

c         N  S  G  Y  N  S  D  E  Q  K  T  T  D  T  A  S  L  P  P  K -

        AATCCTGTGAATCCAGTGATCCTGAACAAAGAAACAGTAGCTGTGGCCCTCCTCACCAGC
   1021 ---------+---------+---------+---------+---------+---------+ 1080
        TTAGGACACTTAGGTCACTAGGACTTGTTTCTTTGTCATCGACACCGGGAGGAGTGGTCG

c         S  C  E  S  S  D  P  E  Q  R  N  S  S  C  G  P  P  H  Q  R -

        GGGCTGATCAACGTCCACCCAGGCCAGCAAGTCATCCACAGGCTTCTTTTAATCTGGCCA
   1081 ---------+---------+---------+---------+---------+---------+ 1140
        CCCGACTAGTTGCAGGTGGGTCCGGTCGTTCAGTAGGTGTCCGAAGAAAATTAGACCGGT

c         A  D  Q  R  P  P  R  P  A  S  H  P  Q  A  S  F  N  L  A  S -

        GTCCTGAGAAGGTCAGTAATACAACTGTAGTATAG
   1141 ---------+---------+---------+----- 1175
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c         P  E  K  V  S  N  T  T  V  V  *  -
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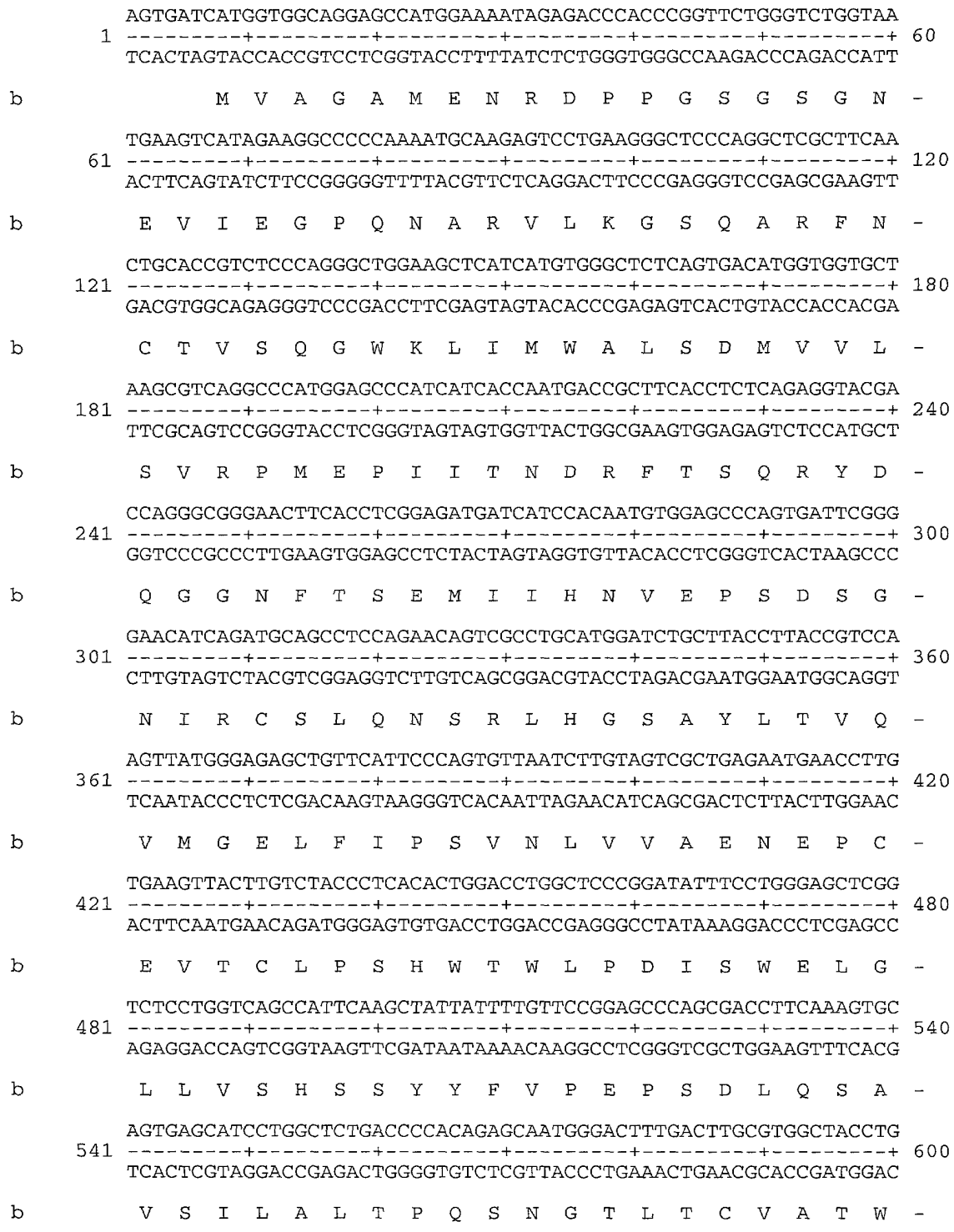
FIGURE 2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1
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b M V A G A M E N R D P P G S G S G N -
TGAAGTCATAGAAGGCCCCCAAAATGCAAGAGTCCTGAAGGGCTCCCAGGCTCGCTTCAA
61
120
ACTTCAGTATCTTCCGGGGGTTTTACGTTCTCAGGACTTCCCGAGGGTCCGAGCGAAGTT
b E V I E G P Q N A R V L K G S Q A R F N -
CTGCACCGTCTCCCAGGGCTGGAAGCTCATCATGTGGGCTCTCAGTGACATGGTGGTGCT
121
180
GACGTGGCAGAGGGTCCCGACCTTCGAGTAGTACACCCGAGAGTCACTGTACCACCACGA
b C T V S Q G W K L I M W A L S D M V V L -
AAGCGTCAGGCCCATGGAGCCCATCATCACCAATGACCGCTTCACCTCTCAGAGGTACGA
181
240
TTCGCAGTCCGGGTACCTCGGGTAGTAGTGGTTACTGGCGAAGTGGAGAGTCTCCATGCT
b S V R P M E P I I T N D R F T S Q R Y D -
CCAGGGCGGGAACTTCACCTCGGAGATGATCATCCACAATGTGGAGCCCAGTGATTCGGG
241
300
GGTCCCGCCCTTGAAGTGGAGCCTCTACTAGTAGGTGTTACACCTCGGGTCACTAAGCCC
b Q G G N F T S E M I I H N V E P S D S G -
GAACATCAGATGCAGCCTCCAGAACAGTCGCCTGCATGGATCTGCTTACCTTACCGTCCA
301
360
CTTGTAGTCTACGTCGGAGGTCTTGTCAGCGGACGTACCTAGACGAATGGAATGGCAGGT
b N I R C S L Q N S R L H G S A Y L T V Q -
AGTTATGGGAGAGCTGTTCATTCCCAGTGTTAATCTTGTAGTCGCTGAGAATGAACCTTG
361
420
TCAATACCCTCTCGACAAGTAAGGGTCACAATTAGAACATCAGCGACTCTTACTTGGAAC
b V M G E L F I P S V N L V V A E N E P C -
TGAAGTTACTTGTCTACCCTCACACTGGACCTGGCTCCCGGATATTTCCTGGGAGCTCGG
421
480
ACTTCAATGAACAGATGGGAGTGTGACCTGGACCGAGGGCCTATAAAGGACCCTCGAGCC
b E V T C L P S H W T W L P D I S W E L G -
TCTCCTGGTCAGCCATTCAAGCTATTATTTTGTTCCGGAGCCCAGCGACCTTCAAAGTGC
481
540
AGAGGACCAGTCGGTAAGTTCGATAATAAAACAAGGCCTCGGGTCGCTGGAAGTTTCACG
b L L V S H S S Y Y F V P E P S D L Q S A -
AGTGAGCATCCTGGCTCTGACCCCACAGAGCAATGGGACTTTGACTTGCGTGGCTACCTG
541
600
TCACTCGTAGGACCGAGACTGGGGTGTCTCGTTACCCTGAAACTGAACGCACCGATGGAC
b V S I L A L T P Q S N G T L T C V A T W -

FIGURE 2 (con't)

```
        GAAGAGCCTGAAGGCCCGCAAGTCTGCAACTGTAAATCTCACTGTGATTCGGTGTCCCCA
    601 ---------+---------+---------+---------+---------+---------+ 660
        CTTCTCGGACTTCCGGGCGTTCAGACGTTGACATTTAGAGTGACACTAAGCCACAGGGGT

b        K  S  L  K  A  R  K  S  A  T  V  N  L  T  V  I  R  C  P  Q  -

        AGACACTGGAGGTGGTATTAATATTCCAGGTGTATTATCAAGTTTACCGAGTTTAGGTTT
    661 ---------+---------+---------+---------+---------+---------+ 720
        TCTGTGACCTCCACCATAATTATAAGGTCCACATAATAGTTCAAATGGCTCAAATCCAAA

b        D  T  G  G  G  I  N  I  P  G  V  L  S  S  L  P  S  L  G  F  -

        TTCATTGCCTACTTGGGGCAAAGTTGGACTTGGACTAGCAGGCACCATGCTTCTGACGCC
    721 ---------+---------+---------+---------+---------+---------+ 780
        AAGTAACGGATGAACCCCGTTTCAACCTGAACCTGATCGTCCGTGGTACGAAGACTGCGG

b        S  L  P  T  W  G  K  V  G  L  G  L  A  G  T  M  L  L  T  P  -

        GACGTGTACTCTTACAATACGCTGCTGCTGCTGCCGCCGTCGTTGTTGTGGCTGCAACTG
    781 ---------+---------+---------+---------+---------+---------+ 840
        CTGCACATGAGAATGTTATGCGACGACGACGACGGCGGCAGCAACAACACCGACGTTGAC

b        T  C  T  L  T  I  R  C  C  C  C  R  R  R  C  C  G  C  N  C  -

        CTGCTGCCGTTGTTGTTTCTGCTGTAGAAGAAAAAGAGGATTTCGTATTCAATTTCAAAA
    841 ---------+---------+---------+---------+---------+---------+ 900
        GACGACGGCAACAACAAAGACGACATCTTCTTTTTCTCCTAAAGCATAAGTTAAAGTTTT

b        C  C  R  C  C  F  C  C  R  R  K  R  G  F  R  I  Q  F  Q  K  -

        GAAATCTGAAAAAGAGAAGACAAACAAAGAAACTGAGACAGAAAGTGGAAATGAAAACTC
    901 ---------+---------+---------+---------+---------+---------+ 960
        CTTTAGACTTTTTCTCTTCTGTTTGTTTCTTTGACTCTGTCTTTCACCTTTACTTTTGAG

b        K  S  E  K  E  K  T  N  K  E  T  E  T  E  S  G  N  E  N  S  -

        CGGCTACAATTCAGATGAACAAAAGACCACAGACACCGCTTCTCTCCCTCCCAAATCCTG
    961 ---------+---------+---------+---------+---------+---------+ 1020
        GCCGATGTTAAGTCTACTTGTTTTCTGGTGTCTGTGGCGAAGAGAGGGAGGGTTTAGGAC

b        G  Y  N  S  D  E  Q  K  T  T  D  T  A  S  L  P  P  K  S  C  -

        TGAATCCAGTGATCCTGAACAAAGAAACAGTAGCTGTGGCCCTCCTCACCAGCGGGCTGA
   1021 ---------+---------+---------+---------+---------+---------+ 1080
        ACTTAGGTCACTAGGACTTGTTTCTTTGTCATCGACACCGGGAGGAGTGGTCGCCCGACT

b        E  S  S  D  P  E  Q  R  N  S  S  C  G  P  P  H  Q  R  A  D  -

        TCAACGTCCACCCAGGCCAGCAAGTCATCCACAGGCTTCTTTTAATCTGGCCAGTCCTGA
   1081 ---------+---------+---------+---------+---------+---------+ 1140
        AGTTGCAGGTGGGTCCGGTCGTTCAGTAGGTGTCCGAAGAAAATTAGACCGGTCAGGACT

b        Q  R  P  P  R  P  A  S  H  P  Q  A  S  F  N  L  A  S  P  E  -

        GAAGGTCAGTAATACAACTGTAGTATAG
   1141 ---------+---------+-------- 1168
        CTTCCAGTCATTATGTTGACATCATATC

b        K  V  S  N  T  T  V  V  *  -
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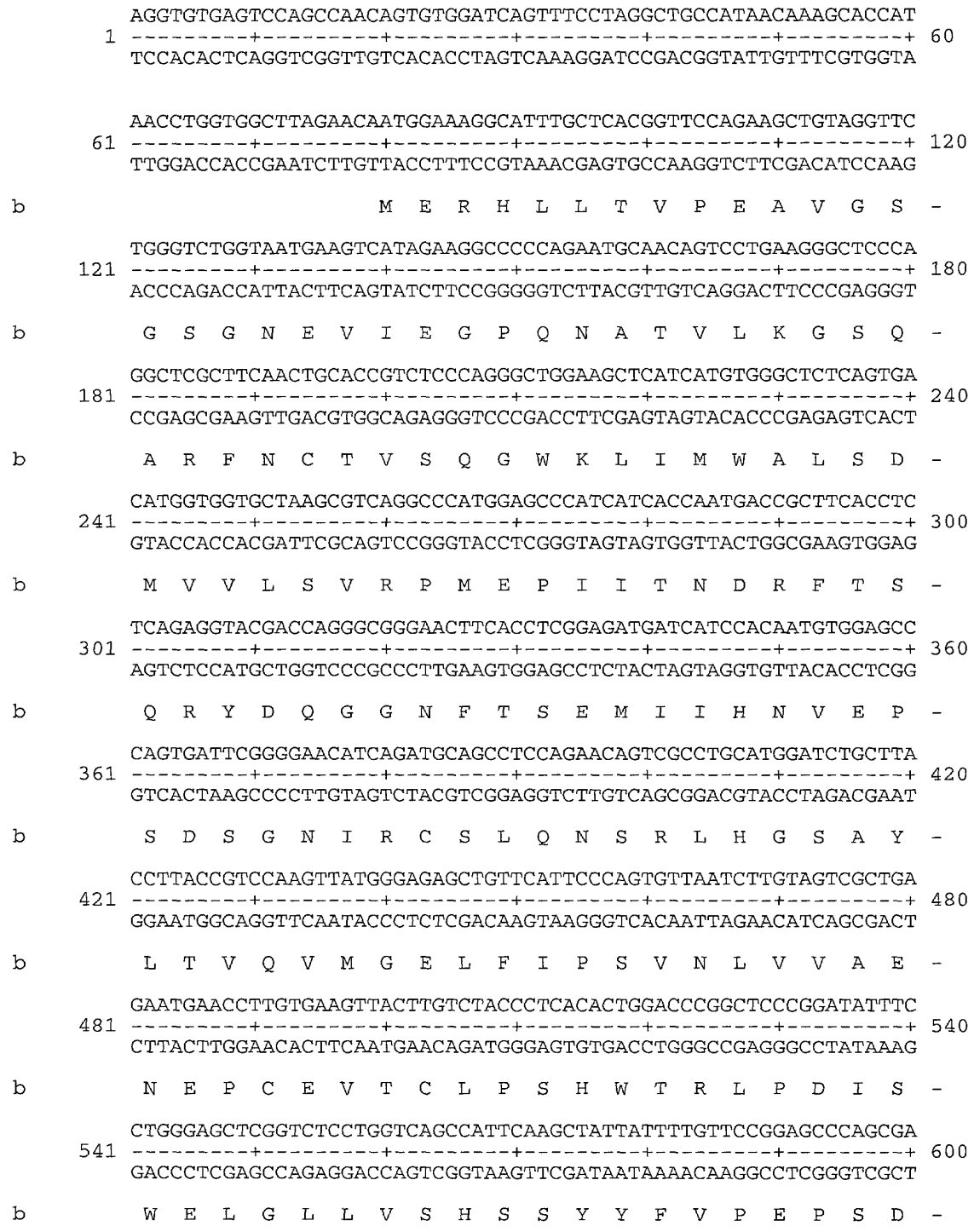
FIGURE 3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61
120
TTGGACCACCGAATCTTGTTACCTTTCCGTAAACGAGTGCCAAGGTCTTCGACATCCAAG
b
M E R H L L T V P E A V G S -
TGGGTCTGGTAATGAAGTCATAGAAGGCCCCCAGAATGCAACAGTCCTGAAGGGCTCCCA
121
180
ACCCAGACCATTACTTCAGTATCTTCCGGGGGTCTTACGTTGTCAGGACTTCCCGAGGGT
b
G S G N E V I E G P Q N A T V L K G S Q -
GGCTCGCTTCAACTGCACCGTCTCCCAGGGCTGGAAGCTCATCATGTGGGCTCTCAGTGA
181
240
CCGAGCGAAGTTGACGTGGCAGAGGGTCCCGACCTTCGAGTAGTACACCCGAGAGTCACT
b
A R F N C T V S Q G W K L I M W A L S D -
CATGGTGGTGCTAAGCGTCAGGCCCATGGAGCCCATCATCACCAATGACCGCTTCACCTC
241
300
GTACCACCACGATTCGCAGTCCGGGTACCTCGGGTAGTAGTGGTTACTGGCGAAGTGGAG
b
M V V L S V R P M E P I I T N D R F T S -
TCAGAGGTACGACCAGGGCGGGAACTTCACCTCGGAGATGATCATCCACAATGTGGAGCC
301
360
AGTCTCCATGCTGGTCCCGCCCTTGAAGTGGAGCCTCTACTAGTAGGTGTTACACCTCGG
b
Q R Y D Q G G N F T S E M I I H N V E P -
CAGTGATTCGGGGAACATCAGATGCAGCCTCCAGAACAGTCGCCTGCATGGATCTGCTTA
361
420
GTCACTAAGCCCCTTGTAGTCTACGTCGGAGGTCTTGTCAGCGGACGTACCTAGACGAAT
b
S D S G N I R C S L Q N S R L H G S A Y -
CCTTACCGTCCAAGTTATGGGAGAGCTGTTCATTCCCAGTGTTAATCTTGTAGTCGCTGA
421
480
GGAATGGCAGGTTCAATACCCTCTCGACAAGTAAGGGTCACAATTAGAACATCAGCGACT
b
L T V Q V M G E L F I P S V N L V V A E -
GAATGAACCTTGTGAAGTTACTTGTCTACCCTCACACTGGACCCGGCTCCCGGATATTTC
481
540
CTTACTTGGAACACTTCAATGAACAGATGGGAGTGTGACCTGGGCCGAGGGCCTATAAAG
b
N E P C E V T C L P S H W T R L P D I S -
CTGGGAGCTCGGTCTCCTGGTCAGCCATTCAAGCTATTATTTTGTTCCGGAGCCCAGCGA
541
600
GACCCTCGAGCCAGAGGACCAGTCGGTAAGTTCGATAATAAAACAAGGCCTCGGGTCGCT
b
W E L G L L V S H S S Y Y F V P E P S D -

FIGURE 3 (con't)

```
          GGCCAGTCCTGAGAAGGTCAGTAATACAACTGTAGTATAG
     1201 ---------+---------+---------+---------+ 1240
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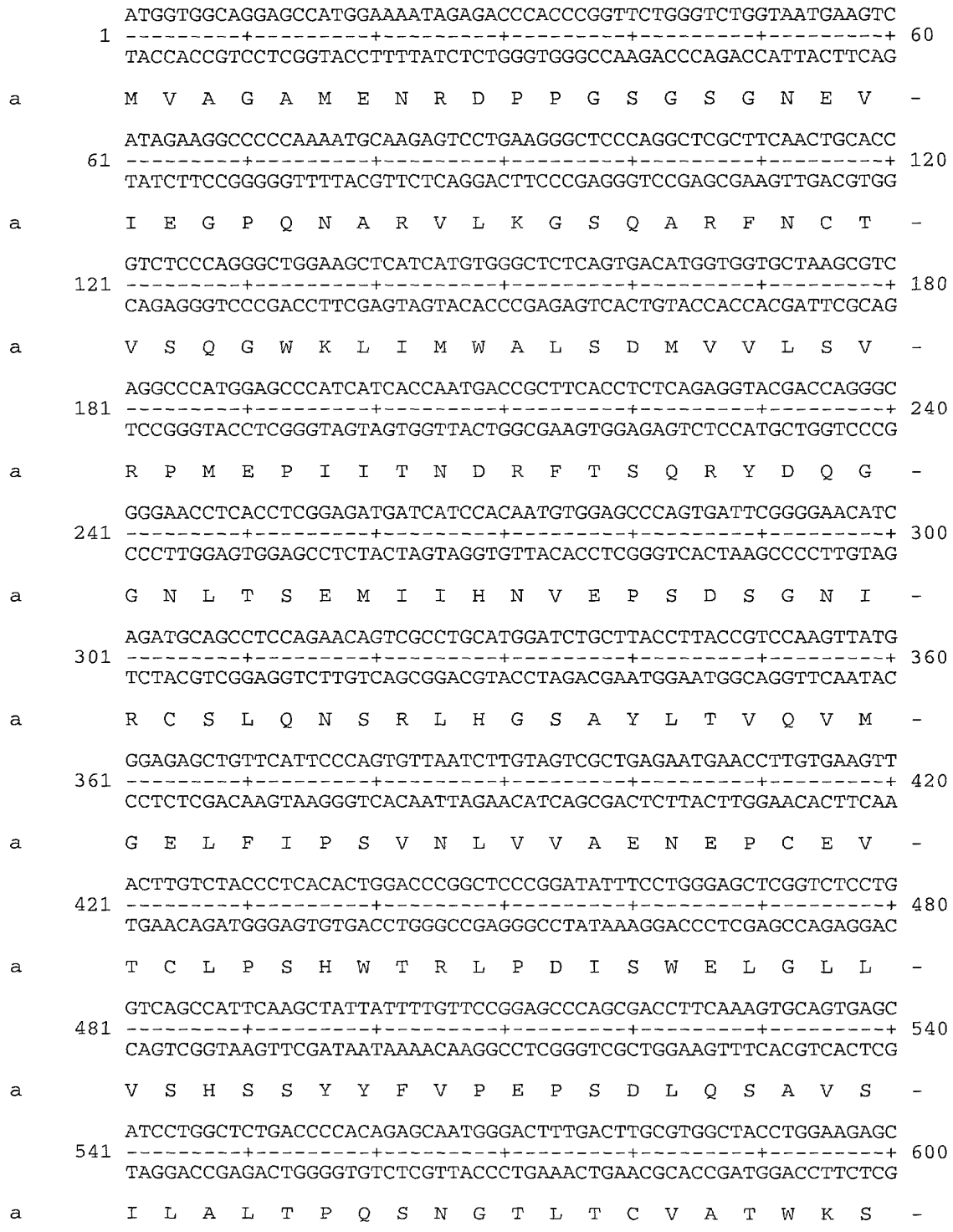
b          A  S  P  E  K  V  S  N  T  T  V  V  *   -
```

FIGURE 4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61
120
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a I E G P Q N A R V L K G S Q A R F N C T -
GTCTCCCAGGGCTGGAAGCTCATCATGTGGGCTCTCAGTGACATGGTGGTGCTAAGCGTC
121
180
CAGAGGGTCCCGACCTTCGAGTAGTACACCCGAGAGTCACTGTACCACCACGATTCGCAG
a V S Q G W K L I M W A L S D M V V L S V -
AGGCCCATGGAGCCCATCATCACCAATGACCGCTTCACCTCTCAGAGGTACGACCAGGGC
181
240
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a R P M E P I I T N D R F T S Q R Y D Q G -
GGGAACCTCACCTCGGAGATGATCATCCACAATGTGGAGCCCAGTGATTCGGGGAACATC
241
300
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a G N L T S E M I I H N V E P S D S G N I -
AGATGCAGCCTCCAGAACAGTCGCCTGCATGGATCTGCTTACCTTACCGTCCAAGTTATG
301
360
TCTACGTCGGAGGTCTTGTCAGCGGACGTACCTAGACGAATGGAATGGCAGGTTCAATAC
a R C S L Q N S R L H G S A Y L T V Q V M -
GGAGAGCTGTTCATTCCCAGTGTTAATCTTGTAGTCGCTGAGAATGAACCTTGTGAAGTT
361
420
CCTCTCGACAAGTAAGGGTCACAATTAGAACATCAGCGACTCTTACTTGGAACACTTCAA
a G E L F I P S V N L V V A E N E P C E V -
ACTTGTCTACCCTCACACTGGACCCGGCTCCCGGATATTTCCTGGGAGCTCGGTCTCCTG
421
480
TGAACAGATGGGAGTGTGACCTGGGCCGAGGGCCTATAAAGGACCCTCGAGCCAGAGGAC
a T C L P S H W T R L P D I S W E L G L L -
GTCAGCCATTCAAGCTATTATTTTGTTCCGGAGCCCAGCGACCTTCAAAGTGCAGTGAGC
481
540
CAGTCGGTAAGTTCGATAATAAAACAAGGCCTCGGGTCGCTGGAAGTTTCACGTCACTCG
a V S H S S Y Y F V P E P S D L Q S A V S -
ATCCTGGCTCTGACCCCACAGAGCAATGGGACTTTGACTTGCGTGGCTACCTGGAAGAGC
541
600
TAGGACCGAGACTGGGGTGTCTCGTTACCCTGAAACTGAACGCACCGATGGACCTTCTCG
a I L A L T P Q S N G T L T C V A T W K S -

FIGURE 4 (con't)

```
       CTGAAGGCCCGCAAGTCTGCAACTGTAAATCTCACTGTGATTCGGTGTCCCCAAGACACT
   601 ---------+---------+---------+---------+---------+---------+ 660
       GACTTCCGGGCGTTCAGACGTTGACATTTAGAGTGACACTAAGCCACAGGGGTTCTGTGA

a          L  K  A  R  K  S  A  T  V  N  L  T  V  I  R  C  P  Q  D  T  -

       GGAGGTGGTATTAATATTCCAGGTGTATTATCAAGTTTACCGAGTTTAGGTTTTTCATTG
   661 ---------+---------+---------+---------+---------+---------+ 720
       CCTCCACCATAATTATAAGGTCCACATAATAGTTCAAATGGCTCAAATCCAAAAAGTAAC

a          G  G  G  I  N  I  P  G  V  L  S  S  L  P  S  L  G  F  S  L  -

       CCTACTTGGGGCAAAGTTGGACTTGGACTAGCAGGCACCATGCTTCTGACGCCGACGTGT
   721 ---------+---------+---------+---------+---------+---------+ 780
       GGATGAACCCCGTTTCAACCTGAACCTGATCGTCCGTGGTACGAAGACTGCGGCTGCACA

a          P  T  W  G  K  V  G  L  G  L  A  G  T  M  L  L  T  P  T  C  -

       ACTCTTACAATACGCTGCTGCTGCTGCCGCCGTCGTTGTTGTGGCTGCAACTGCTGCTGC
   781 ---------+---------+---------+---------+---------+---------+ 840
       TGAGAATGTTATGCGACGACGACGACGGCGGCAGCAACAACACCGACGTTGACGACGACG

a          T  L  T  I  R  C  C  C  C  R  R  R  C  C  G  C  N  C  C  C  -

       CGTTGTTGTTTCTGCTGTAGAAGAAAAAGAGGAAATCTGAAAAAGAGAAGACAAACAAAG
   841 ---------+---------+---------+---------+---------+---------+ 900
       GCAACAACAAAGACGACATCTTCTTTTTCTCCTTTAGACTTTTTCTCTTCTGTTTGTTTC

a          R  C  C  F  C  C  R  R  K  R  G  N  L  K  K  R  R  Q  T  K  -

       AAACTGAGACAGAAAGTGGAAATGAAAACTCCGGCTACAATTCAGATGAACAAAAGACCA
   901 ---------+---------+---------+---------+---------+---------+ 960
       TTTGACTCTGTCTTTCACCTTTACTTTTGAGGCCGATGTTAAGTCTACTTGTTTTCTGGT

a          K  L  R  Q  K  V  E  M  K  T  P  A  T  I  Q  M  N  K  R  P  -

       CAGACACCGCTTCTCTCCCTCCCAAATCCTGTGAATCCAGTGATCCTGAACAAAGAAACA
   961 ---------+---------+---------+---------+---------+---------+ 1020
       GTCTGTGGCGAAGAGAGGGAGGGTTTAGGACACTTAGGTCACTAGGACTTGTTTCTTTGT

a          Q  T  P  L  L  S  L  P  N  P  V  N  P  V  I  L  N  K  E  T  -

       GTAGCTGTGGCCCTCCTCACCAGCGGGCTGATCAACGTCCACCCAGGCCAGCAAGTCATC
  1021 ---------+---------+---------+---------+---------+---------+ 1080
       CATCGACACCGGGAGGAGTGGTCGCCCGACTAGTTGCAGGTGGGTCCGGTCGTTCAGTAG

a          V  A  V  A  L  L  T  S  G  L  I  N  V  H  P  G  Q  Q  V  I  -

       CACAGGCTTCTTTTAATCTGGCCAGTCCTGAGAAGGTCAGTAATACAACTGTAGTATAA
  1081 ---------+---------+---------+---------+---------+--------- 1139
       GTGTCCGAAGAAAATTAGACCGGTCAGGACTCTTCCAGTCATTATGTTGACATCATATT

a          H  R  L  L  L  I  W  P  V  L  R  R  S  V  I  Q  L  *
```

FIGURE 5

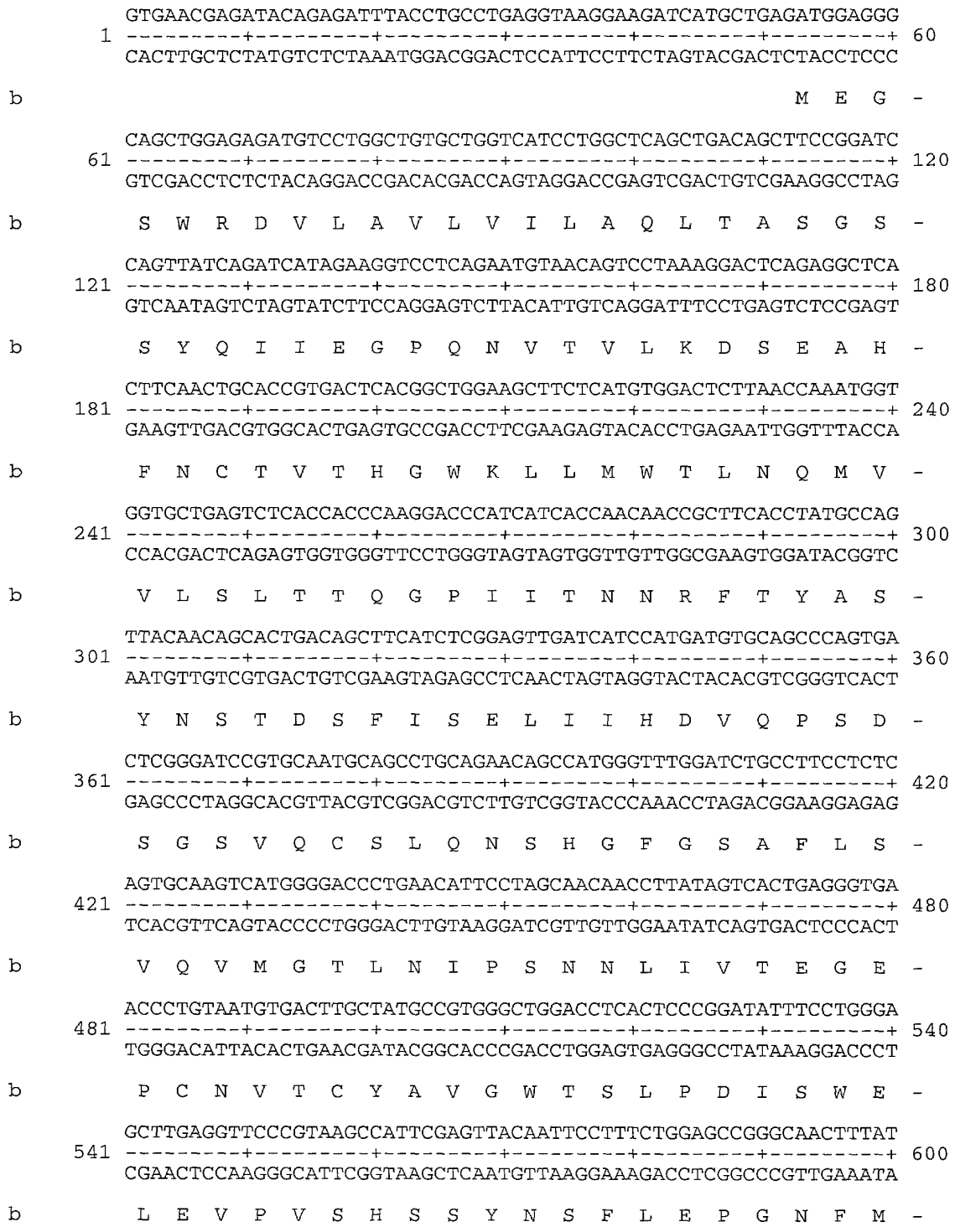
GTGAACGAGATACAGAGATTTACCTGCCTGAGGTAAGGAAGATCATGCTGAGATGGAGGG
1 ---------+---------+---------+---------+---------+---------+ 60
CACTTGCTCTATGTCTCTAAATGGACGGACTCCATTCCTTCTAGTACGACTCTACCTCCC
b M E G -
CAGCTGGAGAGATGTCCTGGCTGTGCTGGTCATCCTGGCTCAGCTGACAGCTTCCGGATC
61 ---------+---------+---------+---------+---------+---------+ 120
GTCGACCTCTCTACAGGACCGACACGACCAGTAGGACCGAGTCGACTGTCGAAGGCCTAG
b S W R D V L A V L V I L A Q L T A S G S -
CAGTTATCAGATCATAGAAGGTCCTCAGAATGTAACAGTCCTAAAGGACTCAGAGGCTCA
121 ---------+---------+---------+---------+---------+---------+ 180
GTCAATAGTCTAGTATCTTCCAGGAGTCTTACATTGTCAGGATTTCCTGAGTCTCCGAGT
b S Y Q I I E G P Q N V T V L K D S E A H -
CTTCAACTGCACCGTGACTCACGGCTGGAAGCTTCTCATGTGGACTCTTAACCAAATGGT
181 ---------+---------+---------+---------+---------+---------+ 240
GAAGTTGACGTGGCACTGAGTGCCGACCTTCGAAGAGTACACCTGAGAATTGGTTTACCA
b F N C T V T H G W K L L M W T L N Q M V -
GGTGCTGAGTCTCACCACCCAAGGACCCATCATCACCAACAACCGCTTCACCTATGCCAG
241 ---------+---------+---------+---------+---------+---------+ 300
CCACGACTCAGAGTGGTGGGTTCCTGGGTAGTAGTGGTTGTTGGCGAAGTGGATACGGTC
b V L S L T T Q G P I I T N N R F T Y A S -
TTACAACAGCACTGACAGCTTCATCTCGGAGTTGATCATCCATGATGTGCAGCCCAGTGA
301 ---------+---------+---------+---------+---------+---------+ 360
AATGTTGTCGTGACTGTCGAAGTAGAGCCTCAACTAGTAGGTACTACACGTCGGGTCACT
b Y N S T D S F I S E L I I H D V Q P S D -
CTCGGGATCCGTGCAATGCAGCCTGCAGAACAGCCATGGGTTTGGATCTGCCTTCCTCTC
361 ---------+---------+---------+---------+---------+---------+ 420
GAGCCCTAGGCACGTTACGTCGGACGTCTTGTCGGTACCCAAACCTAGACGGAAGGAGAG
b S G S V Q C S L Q N S H G F G S A F L S -
AGTGCAAGTCATGGGGACCCTGAACATTCCTAGCAACAACCTTATAGTCACTGAGGGTGA
421 ---------+---------+---------+---------+---------+---------+ 480
TCACGTTCAGTACCCCTGGGACTTGTAAGGATCGTTGTTGGAATATCAGTGACTCCCACT
b V Q V M G T L N I P S N N L I V T E G E -
ACCCTGTAATGTGACTTGCTATGCCGTGGGCTGGACCTCACTCCCGGATATTTCCTGGGA
481 ---------+---------+---------+---------+---------+---------+ 540
TGGGACATTACACTGAACGATACGGCACCCGACCTGGAGTGAGGGCCTATAAAGGACCCT
b P C N V T C Y A V G W T S L P D I S W E -
GCTTGAGGTTCCCGTAAGCCATTCGAGTTACAATTCCTTTCTGGAGCCGGGCAACTTTAT
541 ---------+---------+---------+---------+---------+---------+ 600
CGAACTCCAAGGGCATTCGGTAAGCTCAATGTTAAGGAAAGACCTCGGCCCGTTGAAATA
b L E V P V S H S S Y N S F L E P G N F M -

FIGURE 5 (con't)

```
        GAGGGTCTTGAGTGTCCTGGACCTCACACCACTGGGCAACGGGACCTTGACTTGTGTGGC
    601 ---------+---------+---------+---------+---------+---------+ 660
        CTCCCAGAACTCACAGGACCTGGAGTGTGGTGACCCGTTGCCCTGGAACTGAACACACCG

b        R  V  L  S  V  L  D  L  T  P  L  G  N  G  T  L  T  C  V  A  -

        AGAGCTGAAGGACTTGCAGGCCAGCAAGTCCTTAACTGTCAACCTGACTGTGGTTCAGCC
    661 ---------+---------+---------+---------+---------+---------+ 720
        TCTCGACTTCCTGAACGTCCGGTCGTTCAGGAATTGACAGTTGGACTGACACCAAGTCGG

b        E  L  K  D  L  Q  A  S  K  S  L  T  V  N  L  T  V  V  Q  P  -

        TCCACCTGACAGTATTGGAGAGGAAGGCCCAGCACTGCCGACCTGGGCCATCATCCTGCT
    721 ---------+---------+---------+---------+---------+---------+ 780
        AGGTGGACTGTCATAACCTCTCCTTCCGGGTCGTGACGGCTGGACCCGGTAGTAGGACGA

b        P  P  D  S  I  G  E  E  G  P  A  L  P  T  W  A  I  I  L  L  -

        GGCAGTGGCCTTTTCCTTGCTCTTGATCCTGATCATTGTTTTGATTATAATATTCTGTTG
    781 ---------+---------+---------+---------+---------+---------+ 840
        CCGTCACCGGAAAAGGAACGAGAACTAGGACTAGTAACAAAACTAATATTATAAGACAAC

b        A  V  A  F  S  L  L  L  I  L  I  I  V  L  I  I  I  F  C  C  -

        CTGTTGTGCCTCCAGGAGAGAAAAGGAAGAATCTACTTATCAAAATGAAATAAGGAAATC
    841 ---------+---------+---------+---------+---------+---------+ 900
        GACAACACGGAGGTCCTCTCTTTTCCTTCTTAGATGAATAGTTTTACTTTATTCCTTTAG

b        C  C  A  S  R  R  E  K  E  E  S  T  Y  Q  N  E  I  R  K  S  -

        TGCAAACATGAGGACAAACAAAGCAGATCCGGAGACAAAGTTAAAAAGTGGAAAGGAAAA
    901 ---------+---------+---------+---------+---------+---------+ 960
        ACGTTTGTACTCCTGTTTGTTTCGTCTAGGCCTCTGTTTCAATTTTTCACCTTTCCTTTT

b        A  N  M  R  T  N  K  A  D  P  E  T  K  L  K  S  G  K  E  N  -

        CTACGGGTACAGTTCGGATGAGGCAAAGGCTGCACAGACTGCATCTCTCCCTCCTAAATC
    961 ---------+---------+---------+---------+---------+---------+ 1020
        GATGCCCATGTCAAGCCTACTCCGTTTCCGACGTGTCTGACGTAGAGAGGGAGGATTTAG

b        Y  G  Y  S  S  D  E  A  K  A  A  Q  T  A  S  L  P  P  K  S  -

        TGCTGAAGTCAGCCTTCCAGAAAAACGCAGCAGTAGCCTTCCTTATCAGGAACTCAATAA
   1021 ---------+---------+---------+---------+---------+---------+ 1080
        ACGACTTCAGTCGGAAGGTCTTTTTGCGTCGTCATCGGAAGGAATAGTCCTTGAGTTATT

b        A  E  V  S  L  P  E  K  R  S  S  S  L  P  Y  Q  E  L  N  K  -

        ACATCAGCCCGGTCCAGCAACTCATCCACGGGTTTCCTTTGACATCGCCAGTCCTCAGAA
   1081 ---------+---------+---------+---------+---------+---------+ 1140
        TGTAGTCGGGCCAGGTCGTTGAGTAGGTGCCCAAAGGAAACTGTAGCGGTCAGGAGTCTT

b        H  Q  P  G  P  A  T  H  P  R  V  S  F  D  I  A  S  P  Q  K  -

        GGTCAGAAATGTGACTTTAGTGTAATAAAGACTTCTCATGACTGTACTTGGTGCA
   1141 ---------+---------+---------+---------+---------+----- 1195
        CCAGTCTTTACACTGAAATCACATTATTTCTGAAGAGTACTGACATGAACCACGT

b        V  R  N  V  T  L  V  *
```

FIGURE 6

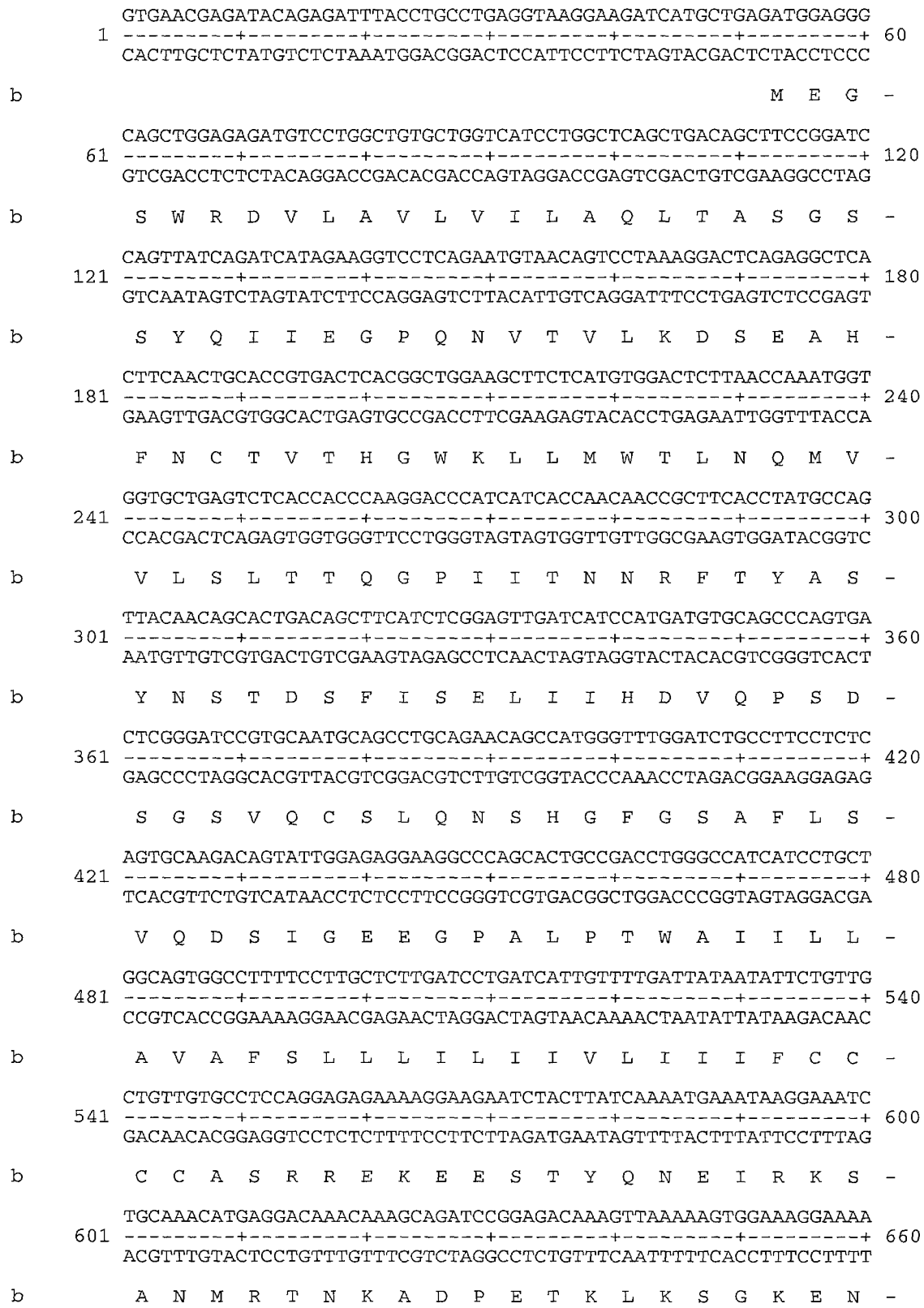
GTGAACGAGATACAGAGATTTACCTGCCTGAGGTAAGGAAGATCATGCTGAGATGGAGGG
1
60
CACTTGCTCTATGTCTCTAAATGGACGGACTCCATTCCTTCTAGTACGACTCTACCTCCC
b
M E G -
CAGCTGGAGAGATGTCCTGGCTGTGCTGGTCATCCTGGCTCAGCTGACAGCTTCCGGATC
61
120
GTCGACCTCTCTACAGGACCGACACGACCAGTAGGACCGAGTCGACTGTCGAAGGCCTAG
b
S W R D V L A V L V I L A Q L T A S G S -
CAGTTATCAGATCATAGAAGGTCCTCAGAATGTAACAGTCCTAAAGGACTCAGAGGCTCA
121
180
GTCAATAGTCTAGTATCTTCCAGGAGTCTTACATTGTCAGGATTTCCTGAGTCTCCGAGT
b
S Y Q I I E G P Q N V T V L K D S E A H -
CTTCAACTGCACCGTGACTCACGGCTGGAAGCTTCTCATGTGGACTCTTAACCAAATGGT
181
240
GAAGTTGACGTGGCACTGAGTGCCGACCTTCGAAGAGTACACCTGAGAATTGGTTTACCA
b
F N C T V T H G W K L L M W T L N Q M V -
GGTGCTGAGTCTCACCACCCAAGGACCCATCATCACCAACAACCGCTTCACCTATGCCAG
241
300
CCACGACTCAGAGTGGTGGGTTCCTGGGTAGTAGTGGTTGTTGGCGAAGTGGATACGGTC
b
V L S L T T Q G P I I T N N R F T Y A S -
TTACAACAGCACTGACAGCTTCATCTCGGAGTTGATCATCCATGATGTGCAGCCCAGTGA
301
360
AATGTTGTCGTGACTGTCGAAGTAGAGCCTCAACTAGTAGGTACTACACGTCGGGTCACT
b
Y N S T D S F I S E L I I H D V Q P S D -
CTCGGGATCCGTGCAATGCAGCCTGCAGAACAGCCATGGGTTTGGATCTGCCTTCCTCTC
361
420
GAGCCCTAGGCACGTTACGTCGGACGTCTTGTCGGTACCCAAACCTAGACGGAAGGAGAG
b
S G S V Q C S L Q N S H G F G S A F L S -
AGTGCAAGACAGTATTGGAGAGGAAGGCCCAGCACTGCCGACCTGGGCCATCATCCTGCT
421
480
TCACGTTCTGTCATAACCTCTCCTTCCGGGTCGTGACGGCTGGACCCGGTAGTAGGACGA
b
V Q D S I G E E G P A L P T W A I I L L -
GGCAGTGGCCTTTTCCTTGCTCTTGATCCTGATCATTGTTTTGATTATAATATTCTGTTG
481
540
CCGTCACCGGAAAAGGAACGAGAACTAGGACTAGTAACAAAACTAATATTATAAGACAAC
b
A V A F S L L L I L I I V L I I I F C C -
CTGTTGTGCCTCCAGGAGAGAAAAGGAAGAATCTACTTATCAAAATGAAATAAGGAAATC
541
600
GACAACACGGAGGTCCTCTCTTTTCCTTCTTAGATGAATAGTTTTACTTTATTCCTTTAG
b
C C A S R R E K E E S T Y Q N E I R K S -
TGCAAACATGAGGACAAACAAAGCAGATCCGGAGACAAAGTTAAAAAGTGGAAAGGAAAA
601
660
ACGTTTGTACTCCTGTTTGTTTCGTCTAGGCCTCTGTTTCAATTTTTCACCTTTCCTTTT
b
A N M R T N K A D P E T K L K S G K E N -

FIGURE 3 (con't)

```
        CCTTCAAAGTGCAGTGAGCATCCTGGCTCTGACCCCACAGAGCAATGGGACTTTGACTTG
    601 ---------+---------+---------+---------+---------+---------+ 660
        GGAAGTTTCACGTCACTCGTAGGACCGAGACTGGGGTGTCTCGTTACCCTGAAACTGAAC

b        L  Q  S  A  V  S  I  L  A  L  T  P  Q  S  N  G  T  L  T  C  -

        CGTGGCTACCTGGAAGAGCCTGAAGGCCCGCAAGTCTGCAACTGTAAATCTCACTGTGAT
    661 ---------+---------+---------+---------+---------+---------+ 720
        GCACCGATGGACCTTCTCGGACTTCCGGGCGTTCAGACGTTGACATTTAGAGTGACACTA

b        V  A  T  W  K  S  L  K  A  R  K  S  A  T  V  N  L  T  V  I  -

        TCGGTGTCCCCAAGACACTGGAGGTGGTATTAATATTCCAGGTGTATTATCAAGTTTACC
    721 ---------+---------+---------+---------+---------+---------+ 780
        AGCCACAGGGGTTCTGTGACCTCCACCATAATTATAAGGTCCACATAATAGTTCAAATGG

b        R  C  P  Q  D  T  G  G  G  I  N  I  P  G  V  L  S  S  L  P  -

        GAGTTTAGGTTTTTCATTGCCTACTTGGGGCAAAGTTGGACTTGGACTAGCAGGCACCAT
    781 ---------+---------+---------+---------+---------+---------+ 840
        CTCAAATCCAAAAAGTAACGGATGAACCCCGTTTCAACCTGAACCTGATCGTCCGTGGTA

b        S  L  G  F  S  L  P  T  W  G  K  V  G  L  G  L  A  G  T  M  -

        GCTTCTGACGCCGACGTGTACTCTTACAATACGCTGCTGCTGCTGCCGCCGTCGTTGTTG
    841 ---------+---------+---------+---------+---------+---------+ 900
        CGAAGACTGCGGCTGCACATGAGAATGTTATGCGACGACGACGACGGCGGCAGCAACAAC

b        L  L  T  P  T  C  T  L  T  I  R  C  C  C  C  R  R  R  C  C  -

        TGGCTGCAACTGCTGCTGCCGTTGTTGTTTCTGCTGTAGAAGAAAAAGAGGATTTCGTAT
    901 ---------+---------+---------+---------+---------+---------+ 960
        ACCGACGTTGACGACGACGGCAACAACAAAGACGACATCTTCTTTTTCTCCTAAAGCATA

b        G  C  N  C  C  C  R  C  C  F  C  C  R  R  K  R  G  F  R  I  -

        TCAATTTCAAAAGAAATCTGAAAAAGAGAAGACAAACAAAGAAACTGAGACAGAAAGTGG
    961 ---------+---------+---------+---------+---------+---------+ 1020
        AGTTAAAGTTTTCTTTAGACTTTTTCTCTTCTGTTTGTTTCTTTGACTCTGTCTTTCACC

b        Q  F  Q  K  K  S  E  K  E  K  T  N  K  E  T  E  T  E  S  G  -

        AAATGAAAACTCCGGCTACAATTCAGATGAACAAAAGACCACAGAAACCGCTTCTCTCCC
   1021 ---------+---------+---------+---------+---------+---------+ 1080
        TTTACTTTTGAGGCCGATGTTAAGTCTACTTGTTTTCTGGTGTCTTTGGCGAAGAGAGGG

b        N  E  N  S  G  Y  N  S  D  E  Q  K  T  T  E  T  A  S  L  P  -

        TCCCAAATCCTGTGAATCCAGTGATCCTGAACAAAGAAACAGTAGCTGTGGCCCTCCTCA
   1081 ---------+---------+---------+---------+---------+---------+ 1140
        AGGGTTTAGGACACTTAGGTCACTAGGACTTGTTTCTTTGTCATCGACACCGGGAGGAGT

b        P  K  S  C  E  S  S  D  P  E  Q  R  N  S  S  C  G  P  P  H  -

        CCAGCGGGCTGATCAACGTCCACCCAGGCCAGCAAGTCATCCACAGGCTTCTTTTAATCT
   1141 ---------+---------+---------+---------+---------+---------+ 1200
        GGTCGCCCGACTAGTTGCAGGTGGGTCCGGTCGTTCAGTAGGTGTCCGAAGAAAATTAGA

b        Q  R  A  D  Q  R  P  P  R  P  A  S  H  P  Q  A  S  F  N  L  -
```

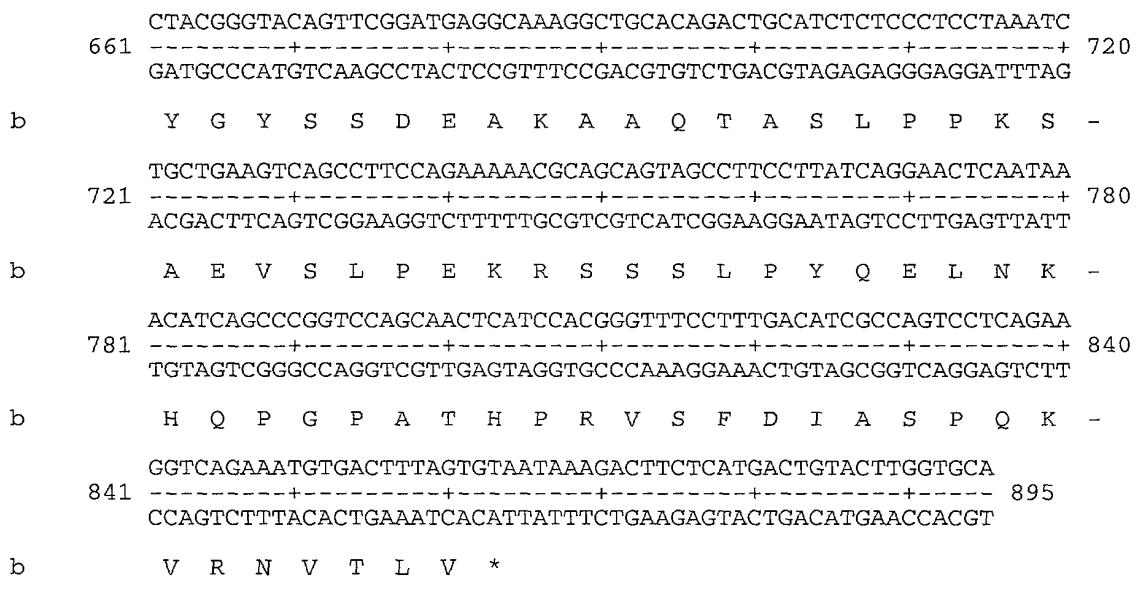
CTACGGGTACAGTTCGGATGAGGCAAAGGCTGCACAGACTGCATCTCTCCCTCCTAAATC
661
GATGCCCATGTCAAGCCTACTCCGTTTCCGACGTGTCTGACGTAGAGAGGGAGGATTTAG
720
b Y G Y S S D E A K A A Q T A S L P P K S -
TGCTGAAGTCAGCCTTCCAGAAAAACGCAGCAGTAGCCTTCCTTATCAGGAACTCAATAA
721
ACGACTTCAGTCGGAAGGTCTTTTTGCGTCGTCATCGGAAGGAATAGTCCTTGAGTTATT
780
b A E V S L P E K R S S S L P Y Q E L N K -
ACATCAGCCCGGTCCAGCAACTCATCCACGGGTTTCCTTTGACATCGCCAGTCCTCAGAA
781
TGTAGTCGGGCCAGGTCGTTGAGTAGGTGCCCAAAGGAAACTGTAGCGGTCAGGAGTCTT
840
b H Q P G P A T H P R V S F D I A S P Q K -
GGTCAGAAATGTGACTTTAGTGTAATAAAGACTTCTCATGACTGTACTTGGTGCA
841
CCAGTCTTTACACTGAAATCACATTATTTCTGAAGAGTACTGACATGAACCACGT
895
b V R N V T L V *

FIGURE 7

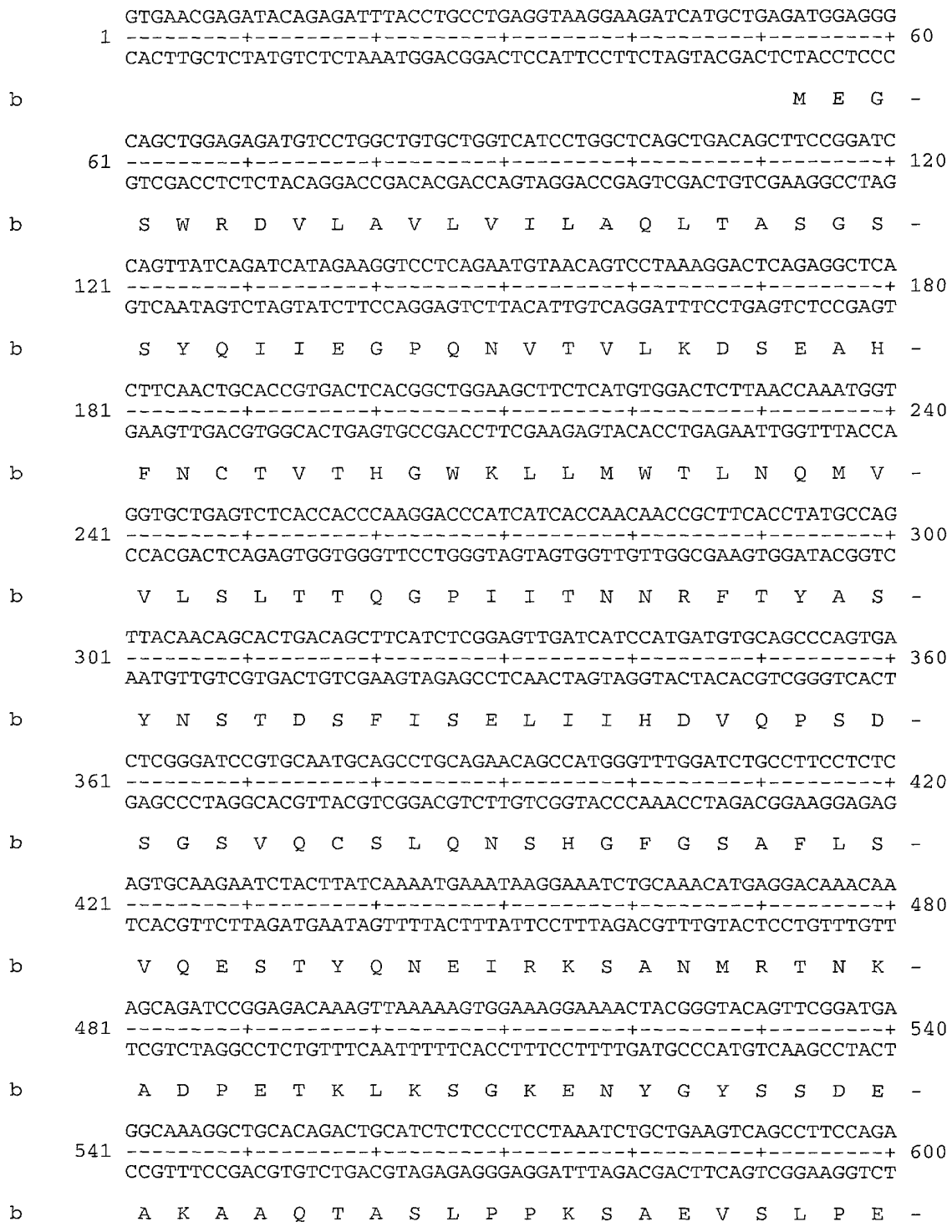
GTGAACGAGATACAGAGATTTACCTGCCTGAGGTAAGGAAGATCATGCTGAGATGGAGGG
1 ---------+---------+---------+---------+---------+---------+ 60
CACTTGCTCTATGTCTCTAAATGGACGGACTCCATTCCTTCTAGTACGACTCTACCTCCC
b M E G -
CAGCTGGAGAGATGTCCTGGCTGTGCTGGTCATCCTGGCTCAGCTGACAGCTTCCGGATC
61 ---------+---------+---------+---------+---------+---------+ 120
GTCGACCTCTCTACAGGACCGACACGACCAGTAGGACCGAGTCGACTGTCGAAGGCCTAG
b S W R D V L A V L V I L A Q L T A S G S -
CAGTTATCAGATCATAGAAGGTCCTCAGAATGTAACAGTCCTAAAGGACTCAGAGGCTCA
121 ---------+---------+---------+---------+---------+---------+ 180
GTCAATAGTCTAGTATCTTCCAGGAGTCTTACATTGTCAGGATTTCCTGAGTCTCCGAGT
b S Y Q I I E G P Q N V T V L K D S E A H -
CTTCAACTGCACCGTGACTCACGGCTGGAAGCTTCTCATGTGGACTCTTAACCAAATGGT
181 ---------+---------+---------+---------+---------+---------+ 240
GAAGTTGACGTGGCACTGAGTGCCGACCTTCGAAGAGTACACCTGAGAATTGGTTTACCA
b F N C T V T H G W K L L M W T L N Q M V -
GGTGCTGAGTCTCACCACCCAAGGACCCATCATCACCAACAACCGCTTCACCTATGCCAG
241 ---------+---------+---------+---------+---------+---------+ 300
CCACGACTCAGAGTGGTGGGTTCCTGGGTAGTAGTGGTTGTTGGCGAAGTGGATACGGTC
b V L S L T T Q G P I I T N N R F T Y A S -
TTACAACAGCACTGACAGCTTCATCTCGGAGTTGATCATCCATGATGTGCAGCCCAGTGA
301 ---------+---------+---------+---------+---------+---------+ 360
AATGTTGTCGTGACTGTCGAAGTAGAGCCTCAACTAGTAGGTACTACACGTCGGGTCACT
b Y N S T D S F I S E L I I H D V Q P S D -
CTCGGGATCCGTGCAATGCAGCCTGCAGAACAGCCATGGGTTTGGATCTGCCTTCCTCTC
361 ---------+---------+---------+---------+---------+---------+ 420
GAGCCCTAGGCACGTTACGTCGGACGTCTTGTCGGTACCCAAACCTAGACGGAAGGAGAG
b S G S V Q C S L Q N S H G F G S A F L S -
AGTGCAAGAATCTACTTATCAAAATGAAATAAGGAAATCTGCAAACATGAGGACAAACAA
421 ---------+---------+---------+---------+---------+---------+ 480
TCACGTTCTTAGATGAATAGTTTTACTTTATTCCTTTAGACGTTTGTACTCCTGTTTGTT
b V Q E S T Y Q N E I R K S A N M R T N K -
AGCAGATCCGGAGACAAAGTTAAAAAGTGGAAAGGAAAACTACGGGTACAGTTCGGATGA
481 ---------+---------+---------+---------+---------+---------+ 540
TCGTCTAGGCCTCTGTTTCAATTTTTCACCTTTCCTTTTGATGCCCATGTCAAGCCTACT
b A D P E T K L K S G K E N Y G Y S S D E -
GGCAAAGGCTGCACAGACTGCATCTCTCCCTCCTAAATCTGCTGAAGTCAGCCTTCCAGA
541 ---------+---------+---------+---------+---------+---------+ 600
CCGTTTCCGACGTGTCTGACGTAGAGAGGGAGGATTTAGACGACTTCAGTCGGAAGGTCT
b A K A A Q T A S L P P K S A E V S L P E -

FIGURE 7 (con't)

```
      AAAACGCAGCAGTAGCCTTCCTTATCAGGAACTCAATAAACATCAGCCCGGTCCAGCAAC
601   ---------+---------+---------+---------+---------+---------+ 660
      TTTTGCGTCGTCATCGGAAGGAATAGTCCTTGAGTTATTTGTAGTCGGGCCAGGTCGTTG

b      K  R  S  S  S  L  P  Y  Q  E  L  N  K  H  Q  P  G  P  A  T  -

      TCATCCACGGGTTTCCTTTGACATCGCCAGTCCTCAGAAGGTCAGAAATGTGACTTTAGT
661   ---------+---------+---------+---------+---------+---------+ 720
      AGTAGGTGCCCAAAGGAAACTGTAGCGGTCAGGAGTCTTCCAGTCTTTACACTGAAATCA

b      H  P  R  V  S  F  D  I  A  S  P  Q  K  V  R  N  V  T  L  V  -

      GTAATAAAGACTTCTCATGACTGTACTTGGTGCA
721   ---------+---------+---------+---- 754
      CATTATTTCTGAAGAGTACTGACATGAACCACGT

b      *
```

FIGURE 8


1 ..................MEGSWRDVLAVLVILAQLTASGSSYQIIEGPQ 32
1 MAYSCQPLQESPLLGFPRLRFIHLFVL.LLVGLLQI.SSGIVGQVSKSVR 48
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189 GINTTISQDPESELYTISSQLDFNATYDHFIDCFIEYGDAHVSQ....NF 234
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235 TWVKPPEDPPDEKQTVPFAWAGPDAVKAIIIFFIAITVIAVIAAIAIIIF 284
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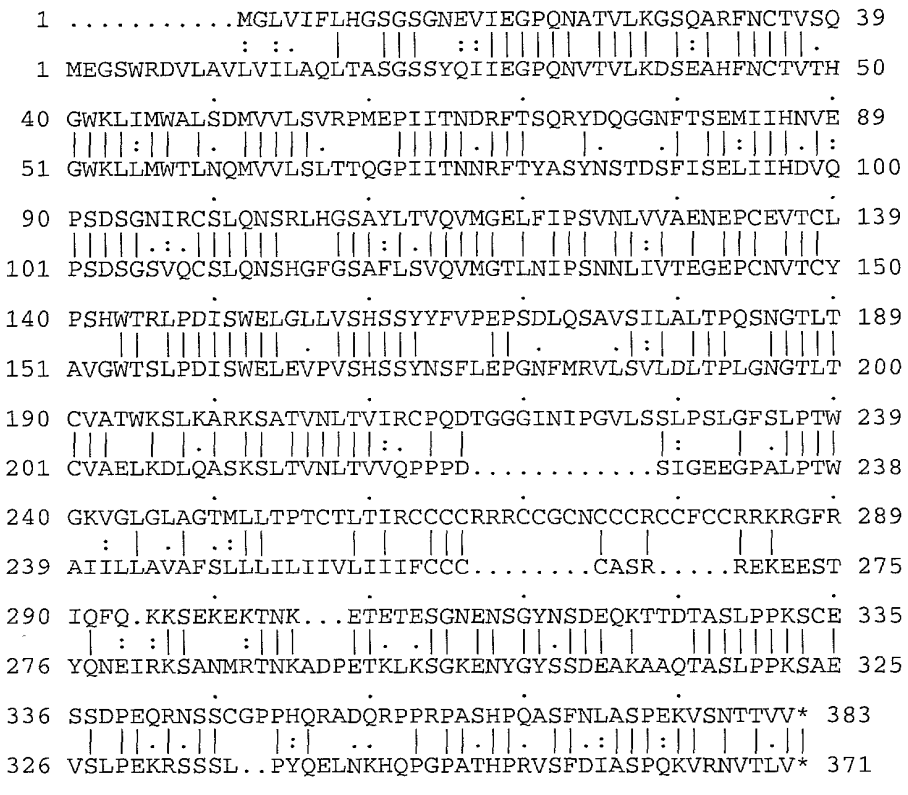
FIGURE 9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326 VSLPEKRSSSL..PYQELNKHQPGPATHPRVSFDIASPQKVRNVTLV* 371

Docket No.: A-692

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

B7-LIKE MOLECULES AND USES THEREOF

which is described and claimed in the specification which:

☒ is attached hereto.

☐ was filed on _____
as Application Serial No. _____
and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56 (a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)			
Country	Application Number	Filing Date (day, month, year)	Priority Claimed

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

PROVISIONAL APPLICATION(S)	
Application Number	Filing Date (day, month, year)
60/214,512	28, June, 2000

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of this application is not disclosed in the prior United States application

DECLARATION AND POWER OF ATTORNEY (cont'd)

in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the filing date of this application:

PRIOR U.S. APPLICATIONS		
APPLICATION SERIAL NO.	FILING DATE	STATUS

Power of Attorney: As a named inventor I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Steven M. Odre (Reg. No. 29,094), Ron K. Levy (Reg. No. 31,539), Scott N. Bernstein (Reg. No. 38,827), Joseph W. Bulock (Reg. No. 37,103), Robert R. Cook (Reg. No. 31,602), Monique L. Cordray (Reg. No. 34,802), Craig A. Crandall (Reg. No. 38,416), Daniel R. Curry (Reg. No. 32,727), Joan D. Eggert (Reg. No. 32,980), Timothy J. Gaul (Reg. No. 33,111), Matthew W. Knight (Reg. No. 36,846), Richard J. Mazza (Reg. No. 27,657), Karen L. Nicastro (Reg. No. 35,968), Nancy A. Oleski (Reg. No. 34,688), Karol M. Pessin (Reg. No. 34,899), Frank S. Ungemach (Reg. No. 34,449), Stuart L. Watt (Reg. No. 32,511), Wendy A. Whiteford (Reg. No. 36,964), Robert B. Winter (Reg. No. 34,458), Thomas D. Zindrick (Reg. No. 32,185), said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

Please send all future correspondence to:

U.S. Patent Operations/ SNB
Dept. 4300, M/S 27-4-A
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

Direct Telephone Calls To:

Scott N. Bernstein
Attorney for Applicants
Registration No.: 38,827
Phone: (805) 447- 4128

DECLARATION AND POWER OF ATTORNEY (cont'd)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole or First Inventor: Andrew A. Welcher

Inventor's Signature: Andrew A. Welcher Date: 11/27/00

Residence and Post Office Address: 1175 Church Street, Ventura, California 93001
(Address, City, State, Zip Code, Country)

Citizenship: United States

Full Name of Second Joint Inventor, if Any: Ulla M. Sarmiento

Inventor's Signature: _____ Date: _____

Residence and Post Office Address: 11340 Broadview Drive, Moorpark, California 93021
(Address, City, State, Zip Code, Country)

Citizenship: Canada

Full Name of Third Joint Inventor, if Any: Henry J. Schultz

Inventor's Signature: Henry J. Schultz Date: 11/28/00

Residence and Post Office Address: 21827 Parvin Drive, Santa Clarita, California 91350
(Address, City, State, Zip Code, Country)

Citizenship: United States

Full Name of Fourth Joint Inventor, if Any: Hilary T. Chute

Inventor's Signature: Hilary T. Chute Date: 11/27/00

Residence and Post Office Address: 26005-F Alizia Canyon Drive, Calabasas, California 91350
(Address, City, State, Zip Code, Country)

Citizenship: United States

Docket No.: A-692

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

B7-LIKE MOLECULES AND USES THEREOF

which is described and claimed in the specification which:

☒ is attached hereto.

☐ was filed on _____
as Application Serial No. _____
and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

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PROVISIONAL APPLICATION(S)	
Application Number	Filing Date (day, month, year)
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DECLARATION AND POWER OF ATTORNEY (cont'd)

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PRIOR U.S. APPLICATIONS		
APPLICATION SERIAL NO.	FILING DATE	STATUS

Power of Attorney: As a named inventor I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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Direct Telephone Calls To:

Scott N. Bernstein
Attorney for Applicants
Registration No.: 38,827
Phone: (805) 447- 4128

DECLARATION AND POWER OF ATTORNEY (cont'd)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name of Sole or First Inventor: Andrew Welcher

Inventor's Signature: _____ Date: _____

Residence and Post Office Address: 1175 Church Street, Ventura, California 93001
(Address, City, State, Zip Code, Country)

Citizenship: United States

Full Name of Second Joint Inventor, if Any: Ulla M. Sarmiento

Inventor's Signature: Ulla Sarmiento Date: 11/22/00

Residence and Post Office Address: 11340 Broadview Drive, Moorpark, California 93021
(Address, City, State, Zip Code, Country)

Citizenship: Canada

Full Name of Third Joint Inventor, if Any: Henry Schultz

Inventor's Signature: _____ Date: _____

Residence and Post Office Address: 21827 Parvin Drive, Santa Clarita, California 91350
(Address, City, State, Zip Code, Country)

Citizenship: _____

Full Name of Fourth Joint Inventor, if Any: Hilary Chute

Inventor's Signature: _____ Date: _____

Residence and Post Office Address: 26005-F Alizia Canyon Drive, Calabasas, California 91350
(Address, City, State, Zip Code, Country)

Citizenship: _____

SEQUENCE LISTING

<110> WELCHER, ANDREW
SARMIENTO, ULLA
SCHULTZ, HENRY
CHUTE, HILARY

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Cys Leu Pro Ser His Trp Thr Arg Leu Pro Asp Ile Ser Trp Glu Leu
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ggt ctc ctg gtc agc cat tca agc tat tat ttt gtt ccg gag ccc agc 533
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155 160 165

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Asp Leu Gln Ser Ala Val Ser Ile Leu Ala Leu Thr Pro Gln Ser Asn
170 175 180 185

ggg act ttg act tgc gtg gct acc tgg aag agc ctg aag gcc cgc aag 629
Gly Thr Leu Thr Cys Val Ala Thr Trp Lys Ser Leu Lys Ala Arg Lys
190 195 200

tct gca act gta aat ctc act gtg att cgg tgt ccc caa gac act gga 677
Ser Ala Thr Val Asn Leu Thr Val Ile Arg Cys Pro Gln Asp Thr Gly
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220 225 230

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Phe Ser Leu Pro Thr Trp Gly Lys Val Gly Leu Gly Leu Ala Gly Thr
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Phe Asn Cys Thr Val Ser Gln Gly Trp Lys Leu Ile Met Trp Ala Leu
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Ser Asp Met Val Val Leu Ser Val Arg Pro Met Glu Pro Ile Ile Thr
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Ser Glu Met Ile Ile His Asn Val Glu Pro Ser Asp Ser Gly Asn Ile
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Ser Tyr Tyr Phe Val Pro Glu Pro Ser Asp Leu Gln Ser Ala Val Ser
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Thr Leu Thr Ile Arg Cys Cys Cys Cys Arg Arg Arg Cys Cys Gly Cys
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Met Val Ala Gly Ala Met Glu Asn Arg Asp Pro Pro Gly Ser Gly Ser
1               5                   10                  15

Gly Asn Glu Val Ile Glu Gly Pro Gln Asn Ala Arg Val Leu Lys Gly
            20                  25                  30

Ser Gln Ala Arg Phe Asn Cys Thr Val Ser Gln Gly Trp Lys Leu Ile
        35                  40                  45

Met Trp Ala Leu Ser Asp Met Val Val Leu Ser Val Arg Pro Met Glu
    50                  55                  60

Pro Ile Ile Thr Asn Asp Arg Phe Thr Ser Gln Arg Tyr Asp Gln Gly
65                  70                  75                  80

Gly Asn Phe Thr Ser Glu Met Ile Ile His Asn Val Glu Pro Ser Asp
                85                  90                  95

Ser Gly Asn Ile Arg Cys Ser Leu Gln Asn Ser Arg Leu His Gly Ser
            100                 105                 110

Ala Tyr Leu Thr Val Gln Val Met Gly Glu Leu Phe Ile Pro Ser Val
        115                 120                 125
```

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Asn Leu Val Val Ala Glu Asn Glu Pro Cys Glu Val Thr Cys Leu Pro
    130                 135                 140

Ser His Trp Thr Trp Leu Pro Asp Ile Ser Trp Glu Leu Gly Leu Leu
145                 150                 155                 160

Val Ser His Ser Ser Tyr Tyr Phe Val Pro Glu Pro Ser Asp Leu Gln
                165                 170                 175

Ser Ala Val Ser Ile Leu Ala Leu Thr Pro Gln Ser Asn Gly Thr Leu
            180                 185                 190

Thr Cys Val Ala Thr Trp Lys Ser Leu Lys Ala Arg Lys Ser Ala Thr
        195                 200                 205

Val Asn Leu Thr Val Ile Arg Cys Pro Gln Asp Thr Gly Gly Gly Ile
    210                 215                 220

Asn Ile Pro Gly Val Leu Ser Ser Leu Pro Ser Leu Gly Phe Ser Leu
225                 230                 235                 240

Pro Thr Trp Gly Lys Val Gly Leu Gly Leu Ala Gly Thr Met Leu Leu
                245                 250                 255

Thr Pro Thr Cys Thr Leu Thr Ile Arg Cys Cys Cys Cys Arg Arg Arg
            260                 265                 270

Cys Cys Gly Cys Asn Cys Cys Cys Arg Cys Cys Phe Cys Cys Arg Arg
        275                 280                 285

Lys Arg Gly Phe Arg Ile Gln Phe Gln Lys Lys Ser Glu Lys Glu Lys
    290                 295                 300

Thr Asn Lys Glu Thr Glu Thr Glu Ser Gly Asn Glu Asn Ser Gly Tyr
305                 310                 315                 320

Asn Ser Asp Glu Gln Lys Thr Thr Asp Thr Ala Ser Leu Pro Pro Lys
                325                 330                 335

Ser Cys Glu Ser Ser Asp Pro Glu Gln Arg Asn Ser Ser Cys Gly Pro
            340                 345                 350

Pro His Gln Arg Ala Asp Gln Arg Pro Pro Arg Pro Ala Ser His Pro
        355                 360                 365

Gln Ala Ser Phe Asn Leu Ala Ser Pro Glu Lys Val Ser Asn Thr Thr
    370                 375                 380
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Val Val
385

<210> 5
<211> 1240
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (80)..(1237)

<400> 5
aggtgtgagt ccagccaaca gtgtggatca gtttcctagg ctgccataac aaagcaccat 60

aacctggtgg cttagaaca atg gaa agg cat ttg ctc acg gtt cca gaa gct 112
Met Glu Arg His Leu Leu Thr Val Pro Glu Ala
1 5 10

gta ggt tct ggg tct ggt aat gaa gtc ata gaa ggc ccc cag aat gca 160
Val Gly Ser Gly Ser Gly Asn Glu Val Ile Glu Gly Pro Gln Asn Ala
15 20 25

aca gtc ctg aag ggc tcc cag gct cgc ttc aac tgc acc gtc tcc cag 208
Thr Val Leu Lys Gly Ser Gln Ala Arg Phe Asn Cys Thr Val Ser Gln
30 35 40

ggc tgg aag ctc atc atg tgg gct ctc agt gac atg gtg gtg cta agc 256
Gly Trp Lys Leu Ile Met Trp Ala Leu Ser Asp Met Val Val Leu Ser
45 50 55

gtc agg ccc atg gag ccc atc atc acc aat gac cgc ttc acc tct cag 304
Val Arg Pro Met Glu Pro Ile Ile Thr Asn Asp Arg Phe Thr Ser Gln
60 65 70 75

agg tac gac cag ggc ggg aac ttc acc tcg gag atg atc atc cac aat 352
Arg Tyr Asp Gln Gly Gly Asn Phe Thr Ser Glu Met Ile Ile His Asn
80 85 90

gtg gag ccc agt gat tcg ggg aac atc aga tgc agc ctc cag aac agt 400
Val Glu Pro Ser Asp Ser Gly Asn Ile Arg Cys Ser Leu Gln Asn Ser
95 100 105

cgc ctg cat gga tct gct tac ctt acc gtc caa gtt atg gga gag ctg 448
Arg Leu His Gly Ser Ala Tyr Leu Thr Val Gln Val Met Gly Glu Leu
110 115 120

ttc att ccc agt gtt aat ctt gta gtc gct gag aat gaa cct tgt gaa 496
Phe Ile Pro Ser Val Asn Leu Val Val Ala Glu Asn Glu Pro Cys Glu
125 130 135

gtt act tgt cta ccc tca cac tgg acc cgg ctc ccg gat att tcc tgg 544
Val Thr Cys Leu Pro Ser His Trp Thr Arg Leu Pro Asp Ile Ser Trp
140 145 150 155

gag ctc ggt ctc ctg gtc agc cat tca agc tat tat ttt gtt ccg gag 592
Glu Leu Gly Leu Leu Val Ser His Ser Ser Tyr Tyr Phe Val Pro Glu
160 165 170

ccc agc gac ctt caa agt gca gtg agc atc ctg gct ctg acc cca cag 640
Pro Ser Asp Leu Gln Ser Ala Val Ser Ile Leu Ala Leu Thr Pro Gln

```
            175                 180                 185

agc aat ggg act ttg act tgc gtg gct acc tgg aag agc ctg aag gcc      688
Ser Asn Gly Thr Leu Thr Cys Val Ala Thr Trp Lys Ser Leu Lys Ala
        190                 195                 200

cgc aag tct gca act gta aat ctc act gtg att cgg tgt ccc caa gac      736
Arg Lys Ser Ala Thr Val Asn Leu Thr Val Ile Arg Cys Pro Gln Asp
    205                 210                 215

act gga ggt ggt att aat att cca ggt gta tta tca agt tta ccg agt      784
Thr Gly Gly Gly Ile Asn Ile Pro Gly Val Leu Ser Ser Leu Pro Ser
220                 225                 230                 235

tta ggt ttt tca ttg cct act tgg ggc aaa gtt gga ctt gga cta gca      832
Leu Gly Phe Ser Leu Pro Thr Trp Gly Lys Val Gly Leu Gly Leu Ala
                240                 245                 250

ggc acc atg ctt ctg acg ccg acg tgt act ctt aca ata cgc tgc tgc      880
Gly Thr Met Leu Leu Thr Pro Thr Cys Thr Leu Thr Ile Arg Cys Cys
            255                 260                 265

tgc tgc cgc cgt cgt tgt tgt ggc tgc aac tgc tgc tgc cgt tgt tgt      928
Cys Cys Arg Arg Arg Cys Cys Gly Cys Asn Cys Cys Cys Arg Cys Cys
        270                 275                 280

ttc tgc tgt aga aga aaa aga gga ttt cgt att caa ttt caa aag aaa      976
Phe Cys Cys Arg Arg Lys Arg Gly Phe Arg Ile Gln Phe Gln Lys Lys
    285                 290                 295

tct gaa aaa gag aag aca aac aaa gaa act gag aca gaa agt gga aat     1024
Ser Glu Lys Glu Lys Thr Asn Lys Glu Thr Glu Thr Glu Ser Gly Asn
300                 305                 310                 315

gaa aac tcc ggc tac aat tca gat gaa caa aag acc aca gaa acc gct     1072
Glu Asn Ser Gly Tyr Asn Ser Asp Glu Gln Lys Thr Thr Glu Thr Ala
                320                 325                 330

tct ctc cct ccc aaa tcc tgt gaa tcc agt gat cct gaa caa aga aac     1120
Ser Leu Pro Pro Lys Ser Cys Glu Ser Ser Asp Pro Glu Gln Arg Asn
            335                 340                 345

agt agc tgt ggc cct cct cac cag cgg gct gat caa cgt cca ccc agg     1168
Ser Ser Cys Gly Pro Pro His Gln Arg Ala Asp Gln Arg Pro Pro Arg
        350                 355                 360

cca gca agt cat cca cag gct tct ttt aat ctg gcc agt cct gag aag     1216
Pro Ala Ser His Pro Gln Ala Ser Phe Asn Leu Ala Ser Pro Glu Lys
    365                 370                 375

gtc agt aat aca act gta gta tag                                    1240
Val Ser Asn Thr Thr Val Val
380                 385
```

<210> 6
<211> 386
<212> PRT
<213> Homo sapiens

<400> 6

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Met Glu Arg His Leu Leu Thr Val Pro Glu Ala Val Gly Ser Gly Ser
1               5                   10                  15
```

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Gly Asn Glu Val Ile Glu Gly Pro Gln Asn Ala Thr Val Leu Lys Gly
            20                  25                  30

Ser Gln Ala Arg Phe Asn Cys Thr Val Ser Gln Gly Trp Lys Leu Ile
        35                  40                  45

Met Trp Ala Leu Ser Asp Met Val Val Leu Ser Val Arg Pro Met Glu
    50                  55                  60

Pro Ile Ile Thr Asn Asp Arg Phe Thr Ser Gln Arg Tyr Asp Gln Gly
65                  70                  75                  80

Gly Asn Phe Thr Ser Glu Met Ile Ile His Asn Val Glu Pro Ser Asp
                85                  90                  95

Ser Gly Asn Ile Arg Cys Ser Leu Gln Asn Ser Arg Leu His Gly Ser
            100                 105                 110

Ala Tyr Leu Thr Val Gln Val Met Gly Glu Leu Phe Ile Pro Ser Val
        115                 120                 125

Asn Leu Val Val Ala Glu Asn Glu Pro Cys Glu Val Thr Cys Leu Pro
    130                 135                 140

Ser His Trp Thr Arg Leu Pro Asp Ile Ser Trp Glu Leu Gly Leu Leu
145                 150                 155                 160

Val Ser His Ser Ser Tyr Tyr Phe Val Pro Glu Pro Ser Asp Leu Gln
                165                 170                 175

Ser Ala Val Ser Ile Leu Ala Leu Thr Pro Gln Ser Asn Gly Thr Leu
            180                 185                 190

Thr Cys Val Ala Thr Trp Lys Ser Leu Lys Ala Arg Lys Ser Ala Thr
        195                 200                 205

Val Asn Leu Thr Val Ile Arg Cys Pro Gln Asp Thr Gly Gly Gly Ile
    210                 215                 220

Asn Ile Pro Gly Val Leu Ser Ser Leu Pro Ser Leu Gly Phe Ser Leu
225                 230                 235                 240

Pro Thr Trp Gly Lys Val Gly Leu Gly Leu Ala Gly Thr Met Leu Leu
                245                 250                 255

Thr Pro Thr Cys Thr Leu Thr Ile Arg Cys Cys Cys Cys Arg Arg Arg
            260                 265                 270
```

```
Cys Cys Gly Cys Asn Cys Cys Cys Arg Cys Cys Phe Cys Cys Arg Arg
        275                 280                 285

Lys Arg Gly Phe Arg Ile Gln Phe Gln Lys Lys Ser Glu Lys Glu Lys
    290                 295                 300

Thr Asn Lys Glu Thr Glu Thr Glu Ser Gly Asn Glu Asn Ser Gly Tyr
305                 310                 315                 320

Asn Ser Asp Glu Gln Lys Thr Thr Glu Thr Ala Ser Leu Pro Pro Lys
                325                 330                 335

Ser Cys Glu Ser Ser Asp Pro Glu Gln Arg Asn Ser Ser Cys Gly Pro
            340                 345                 350

Pro His Gln Arg Ala Asp Gln Arg Pro Pro Arg Pro Ala Ser His Pro
        355                 360                 365

Gln Ala Ser Phe Asn Leu Ala Ser Pro Glu Lys Val Ser Asn Thr Thr
    370                 375                 380

Val Val
385


<210>  7
<211>  1139
<212>  DNA
<213>  Homo sapiens

<220>
<221>  CDS
<222>  (1)..(1131)

<400>  7
atg gtg gca gga gcc atg gaa aat aga gac cca ccc ggt tct ggg tct      48
Met Val Ala Gly Ala Met Glu Asn Arg Asp Pro Pro Gly Ser Gly Ser
1               5                   10                  15

ggt aat gaa gtc ata gaa ggc ccc caa aat gca aga gtc ctg aag ggc      96
Gly Asn Glu Val Ile Glu Gly Pro Gln Asn Ala Arg Val Leu Lys Gly
            20                  25                  30

tcc cag gct cgc ttc aac tgc acc gtc tcc cag ggc tgg aag ctc atc     144
Ser Gln Ala Arg Phe Asn Cys Thr Val Ser Gln Gly Trp Lys Leu Ile
        35                  40                  45

atg tgg gct ctc agt gac atg gtg gtg cta agc gtc agg ccc atg gag     192
Met Trp Ala Leu Ser Asp Met Val Val Leu Ser Val Arg Pro Met Glu
    50                  55                  60

ccc atc atc acc aat gac cgc ttc acc tct cag agg tac gac cag ggc     240
Pro Ile Ile Thr Asn Asp Arg Phe Thr Ser Gln Arg Tyr Asp Gln Gly
65                  70                  75                  80
```

```
ggg aac ctc acc tcg gag atg atc atc cac aat gtg gag ccc agt gat      288
Gly Asn Leu Thr Ser Glu Met Ile Ile His Asn Val Glu Pro Ser Asp
                85                  90                  95

tcg ggg aac atc aga tgc agc ctc cag aac agt cgc ctg cat gga tct      336
Ser Gly Asn Ile Arg Cys Ser Leu Gln Asn Ser Arg Leu His Gly Ser
            100                 105                 110

gct tac ctt acc gtc caa gtt atg gga gag ctg ttc att ccc agt gtt      384
Ala Tyr Leu Thr Val Gln Val Met Gly Glu Leu Phe Ile Pro Ser Val
        115                 120                 125

aat ctt gta gtc gct gag aat gaa cct tgt gaa gtt act tgt cta ccc      432
Asn Leu Val Val Ala Glu Asn Glu Pro Cys Glu Val Thr Cys Leu Pro
    130                 135                 140

tca cac tgg acc cgg ctc ccg gat att tcc tgg gag ctc ggt ctc ctg      480
Ser His Trp Thr Arg Leu Pro Asp Ile Ser Trp Glu Leu Gly Leu Leu
145                 150                 155                 160

gtc agc cat tca agc tat tat ttt gtt ccg gag ccc agc gac ctt caa      528
Val Ser His Ser Ser Tyr Tyr Phe Val Pro Glu Pro Ser Asp Leu Gln
                165                 170                 175

agt gca gtg agc atc ctg gct ctg acc cca cag agc aat ggg act ttg      576
Ser Ala Val Ser Ile Leu Ala Leu Thr Pro Gln Ser Asn Gly Thr Leu
            180                 185                 190

act tgc gtg gct acc tgg aag agc ctg aag gcc cgc aag tct gca act      624
Thr Cys Val Ala Thr Trp Lys Ser Leu Lys Ala Arg Lys Ser Ala Thr
        195                 200                 205

gta aat ctc act gtg att cgg tgt ccc caa gac act gga ggt ggt att      672
Val Asn Leu Thr Val Ile Arg Cys Pro Gln Asp Thr Gly Gly Gly Ile
    210                 215                 220

aat att cca ggt gta tta tca agt tta ccg agt tta ggt ttt tca ttg      720
Asn Ile Pro Gly Val Leu Ser Ser Leu Pro Ser Leu Gly Phe Ser Leu
225                 230                 235                 240

cct act tgg ggc aaa gtt gga ctt gga cta gca ggc acc atg ctt ctg      768
Pro Thr Trp Gly Lys Val Gly Leu Gly Leu Ala Gly Thr Met Leu Leu
                245                 250                 255

acg ccg acg tgt act ctt aca ata cgc tgc tgc tgc tgc cgc cgt cgt      816
Thr Pro Thr Cys Thr Leu Thr Ile Arg Cys Cys Cys Cys Arg Arg Arg
            260                 265                 270

tgt tgt ggc tgc aac tgc tgc tgc cgt tgt tgt ttc tgc tgt aga aga      864
Cys Cys Gly Cys Asn Cys Cys Cys Arg Cys Cys Phe Cys Cys Arg Arg
        275                 280                 285

aaa aga gga aat ctg aaa aag aga aga caa aca aag aaa ctg aga cag      912
Lys Arg Gly Asn Leu Lys Lys Arg Arg Gln Thr Lys Lys Leu Arg Gln
    290                 295                 300

aaa gtg gaa atg aaa act ccg gct aca att cag atg aac aaa aga cca      960
Lys Val Glu Met Lys Thr Pro Ala Thr Ile Gln Met Asn Lys Arg Pro
305                 310                 315                 320

cag aca ccg ctt ctc tcc ctc cca aat cct gtg aat cca gtg atc ctg     1008
Gln Thr Pro Leu Leu Ser Leu Pro Asn Pro Val Asn Pro Val Ile Leu
                325                 330                 335
```

```
aac aaa gaa aca gta gct gtg gcc ctc ctc acc agc ggg ctg atc aac      1056
Asn Lys Glu Thr Val Ala Val Ala Leu Leu Thr Ser Gly Leu Ile Asn
            340                 345                 350

gtc cac cca ggc cag caa gtc atc cac agg ctt ctt tta atc tgg cca      1104
Val His Pro Gly Gln Gln Val Ile His Arg Leu Leu Leu Ile Trp Pro
        355                 360                 365

gtc ctg aga agg tca gta ata caa ctg tagtataa                        1139
Val Leu Arg Arg Ser Val Ile Gln Leu
    370                 375
```

<210> 8
<211> 377
<212> PRT
<213> Homo sapiens

<400> 8

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Met Val Ala Gly Ala Met Glu Asn Arg Asp Pro Pro Gly Ser Gly Ser
1               5                   10                  15

Gly Asn Glu Val Ile Glu Gly Pro Gln Asn Ala Arg Val Leu Lys Gly
            20                  25                  30

Ser Gln Ala Arg Phe Asn Cys Thr Val Ser Gln Gly Trp Lys Leu Ile
        35                  40                  45

Met Trp Ala Leu Ser Asp Met Val Val Leu Ser Val Arg Pro Met Glu
    50                  55                  60

Pro Ile Ile Thr Asn Asp Arg Phe Thr Ser Gln Arg Tyr Asp Gln Gly
65                  70                  75                  80

Gly Asn Leu Thr Ser Glu Met Ile Ile His Asn Val Glu Pro Ser Asp
                85                  90                  95

Ser Gly Asn Ile Arg Cys Ser Leu Gln Asn Ser Arg Leu His Gly Ser
            100                 105                 110

Ala Tyr Leu Thr Val Gln Val Met Gly Glu Leu Phe Ile Pro Ser Val
        115                 120                 125

Asn Leu Val Val Ala Glu Asn Glu Pro Cys Glu Val Thr Cys Leu Pro
    130                 135                 140

Ser His Trp Thr Arg Leu Pro Asp Ile Ser Trp Glu Leu Gly Leu Leu
145                 150                 155                 160

Val Ser His Ser Ser Tyr Tyr Phe Val Pro Glu Pro Ser Asp Leu Gln
                165                 170                 175
```

```
Ser Ala Val Ser Ile Leu Ala Leu Thr Pro Gln Ser Asn Gly Thr Leu
            180                 185                 190

Thr Cys Val Ala Thr Trp Lys Ser Leu Lys Ala Arg Lys Ser Ala Thr
        195                 200                 205

Val Asn Leu Thr Val Ile Arg Cys Pro Gln Asp Thr Gly Gly Gly Ile
    210                 215                 220

Asn Ile Pro Gly Val Leu Ser Ser Leu Pro Ser Leu Gly Phe Ser Leu
225                 230                 235                 240

Pro Thr Trp Gly Lys Val Gly Leu Gly Leu Ala Gly Thr Met Leu Leu
                245                 250                 255

Thr Pro Thr Cys Thr Leu Thr Ile Arg Cys Cys Cys Cys Arg Arg Arg
            260                 265                 270

Cys Cys Gly Cys Asn Cys Cys Cys Arg Cys Cys Phe Cys Cys Arg Arg
        275                 280                 285

Lys Arg Gly Asn Leu Lys Lys Arg Arg Gln Thr Lys Lys Leu Arg Gln
    290                 295                 300

Lys Val Glu Met Lys Thr Pro Ala Thr Ile Gln Met Asn Lys Arg Pro
305                 310                 315                 320

Gln Thr Pro Leu Leu Ser Leu Pro Asn Pro Val Asn Pro Val Ile Leu
                325                 330                 335

Asn Lys Glu Thr Val Ala Val Ala Leu Leu Thr Ser Gly Leu Ile Asn
            340                 345                 350

Val His Pro Gly Gln Gln Val Ile His Arg Leu Leu Leu Ile Trp Pro
        355                 360                 365

Val Leu Arg Arg Ser Val Ile Gln Leu
    370                 375
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<210> 9
<211> 1195
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (53)..(1162)

<400> 9
gtgaacgaga tacagagatt tacctgcctg aggtaaggaa gatcatgctg ag atg gag 58
Met Glu

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                                                         1

ggc agc tgg aga gat gtc ctg gct gtg ctg gtc atc ctg gct cag ctg       106
Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala Gln Leu
         5                  10                  15

aca gct tcc gga tcc agt tat cag atc ata gaa ggt cct cag aat gta       154
Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln Asn Val
    20                  25                  30

aca gtc cta aag gac tca gag gct cac ttc aac tgc acc gtg act cac       202
Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val Thr His
35                  40                  45                  50

ggc tgg aag ctt ctc atg tgg act ctt aac caa atg gtg gtg ctg agt       250
Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val Leu Ser
                55                  60                  65

ctc acc acc caa gga ccc atc atc acc aac aac cgc ttc acc tat gcc       298
Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Thr Tyr Ala
            70                  75                  80

agt tac aac agc act gac agc ttc atc tcg gag ttg atc atc cat gat       346
Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile His Asp
        85                  90                  95

gtg cag ccc agt gac tcg gga tcc gtg caa tgc agc ctg cag aac agc       394
Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln Asn Ser
    100                 105                 110

cat ggg ttt gga tct gcc ttc ctc tca gtg caa gtc atg ggg acc ctg       442
His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Val Met Gly Thr Leu
115                 120                 125                 130

aac att cct agc aac aac ctt ata gtc act gag ggt gaa ccc tgt aat       490
Asn Ile Pro Ser Asn Asn Leu Ile Val Thr Glu Gly Glu Pro Cys Asn
                135                 140                 145

gtg act tgc tat gcc gtg ggc tgg acc tca ctc ccg gat att tcc tgg       538
Val Thr Cys Tyr Ala Val Gly Trp Thr Ser Leu Pro Asp Ile Ser Trp
            150                 155                 160

gag ctt gag gtt ccc gta agc cat tcg agt tac aat tcc ttt ctg gag       586
Glu Leu Glu Val Pro Val Ser His Ser Ser Tyr Asn Ser Phe Leu Glu
        165                 170                 175

ccg ggc aac ttt atg agg gtc ttg agt gtc ctg gac ctc aca cca ctg       634
Pro Gly Asn Phe Met Arg Val Leu Ser Val Leu Asp Leu Thr Pro Leu
    180                 185                 190

ggc aac ggg acc ttg act tgt gtg gca gag ctg aag gac ttg cag gcc       682
Gly Asn Gly Thr Leu Thr Cys Val Ala Glu Leu Lys Asp Leu Gln Ala
195                 200                 205                 210

agc aag tcc tta act gtc aac ctg act gtg gtt cag cct cca cct gac       730
Ser Lys Ser Leu Thr Val Asn Leu Thr Val Val Gln Pro Pro Pro Asp
                215                 220                 225

agt att gga gag gaa ggc cca gca ctg ccg acc tgg gcc atc atc ctg       778
Ser Ile Gly Glu Glu Gly Pro Ala Leu Pro Thr Trp Ala Ile Ile Leu
            230                 235                 240

ctg gca gtg gcc ttt tcc ttg ctc ttg atc ctg atc att gtt ttg att       826
Leu Ala Val Ala Phe Ser Leu Leu Leu Ile Leu Ile Ile Val Leu Ile
```

```
        245                 250                 255

ata ata ttc tgt tgc tgt tgt gcc tcc agg aga gaa aag gaa gaa tct      874
Ile Ile Phe Cys Cys Cys Cys Ala Ser Arg Arg Glu Lys Glu Glu Ser
    260                 265                 270

act tat caa aat gaa ata agg aaa tct gca aac atg agg aca aac aaa      922
Thr Tyr Gln Asn Glu Ile Arg Lys Ser Ala Asn Met Arg Thr Asn Lys
275                 280                 285                 290

gca gat ccg gag aca aag tta aaa agt gga aag gaa aac tac ggg tac      970
Ala Asp Pro Glu Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr Gly Tyr
                295                 300                 305

agt tcg gat gag gca aag gct gca cag act gca tct ctc cct cct aaa     1018
Ser Ser Asp Glu Ala Lys Ala Ala Gln Thr Ala Ser Leu Pro Pro Lys
            310                 315                 320

tct gct gaa gtc agc ctt cca gaa aaa cgc agc agt agc ctt cct tat     1066
Ser Ala Glu Val Ser Leu Pro Glu Lys Arg Ser Ser Ser Leu Pro Tyr
        325                 330                 335

cag gaa ctc aat aaa cat cag ccc ggt cca gca act cat cca cgg gtt     1114
Gln Glu Leu Asn Lys His Gln Pro Gly Pro Ala Thr His Pro Arg Val
    340                 345                 350

tcc ttt gac atc gcc agt cct cag aag gtc aga aat gtg act tta gtg     1162
Ser Phe Asp Ile Ala Ser Pro Gln Lys Val Arg Asn Val Thr Leu Val
355                 360                 365                 370

taataaagac ttctcatgac tgtacttggt gca                               1195
```

<210> 10
<211> 370
<212> PRT
<213> Mus musculus

<400> 10

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Met Glu Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala
1               5                   10                  15

Gln Leu Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln
            20                  25                  30

Asn Val Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val
        35                  40                  45

Thr His Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val
    50                  55                  60

Leu Ser Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Thr
65                  70                  75                  80

Tyr Ala Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile
                85                  90                  95
```

```
His Asp Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln
            100                 105                 110

Asn Ser His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Val Met Gly
        115                 120                 125

Thr Leu Asn Ile Pro Ser Asn Asn Leu Ile Val Thr Glu Gly Glu Pro
    130                 135                 140

Cys Asn Val Thr Cys Tyr Ala Val Gly Trp Thr Ser Leu Pro Asp Ile
145                 150                 155                 160

Ser Trp Glu Leu Glu Val Pro Val Ser His Ser Ser Tyr Asn Ser Phe
                165                 170                 175

Leu Glu Pro Gly Asn Phe Met Arg Val Leu Ser Val Leu Asp Leu Thr
            180                 185                 190

Pro Leu Gly Asn Gly Thr Leu Thr Cys Val Ala Glu Leu Lys Asp Leu
        195                 200                 205

Gln Ala Ser Lys Ser Leu Thr Val Asn Leu Thr Val Val Gln Pro Pro
    210                 215                 220

Pro Asp Ser Ile Gly Glu Glu Gly Pro Ala Leu Pro Thr Trp Ala Ile
225                 230                 235                 240

Ile Leu Leu Ala Val Ala Phe Ser Leu Leu Leu Ile Leu Ile Ile Val
                245                 250                 255

Leu Ile Ile Ile Phe Cys Cys Cys Cys Ala Ser Arg Arg Glu Lys Glu
            260                 265                 270

Glu Ser Thr Tyr Gln Asn Glu Ile Arg Lys Ser Ala Asn Met Arg Thr
        275                 280                 285

Asn Lys Ala Asp Pro Glu Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr
    290                 295                 300

Gly Tyr Ser Ser Asp Glu Ala Lys Ala Ala Gln Thr Ala Ser Leu Pro
305                 310                 315                 320

Pro Lys Ser Ala Glu Val Ser Leu Pro Glu Lys Arg Ser Ser Ser Leu
                325                 330                 335

Pro Tyr Gln Glu Leu Asn Lys His Gln Pro Gly Pro Ala Thr His Pro
            340                 345                 350
```

```
Arg Val Ser Phe Asp Ile Ala Ser Pro Gln Lys Val Arg Asn Val Thr
        355                 360                 365

Leu Val
    370
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<210> 11
<211> 895
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (53)..(862)

<400> 11

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gtgaacgaga tacagagatt tacctgcctg aggtaaggaa gatcatgctg ag atg gag      58
                                                          Met Glu
                                                          1

ggc agc tgg aga gat gtc ctg gct gtg ctg gtc atc ctg gct cag ctg      106
Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala Gln Leu
        5                   10                  15

aca gct tcc gga tcc agt tat cag atc ata gaa ggt cct cag aat gta      154
Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln Asn Val
    20                  25                  30

aca gtc cta aag gac tca gag gct cac ttc aac tgc acc gtg act cac      202
Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val Thr His
35                  40                  45                  50

ggc tgg aag ctt ctc atg tgg act ctt aac caa atg gtg gtg ctg agt      250
Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val Leu Ser
                55                  60                  65

ctc acc acc caa gga ccc atc atc acc aac aac cgc ttc acc tat gcc      298
Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Thr Tyr Ala
            70                  75                  80

agt tac aac agc act gac agc ttc atc tcg gag ttg atc atc cat gat      346
Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile His Asp
        85                  90                  95

gtg cag ccc agt gac tcg gga tcc gtg caa tgc agc ctg cag aac agc      394
Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln Asn Ser
    100                 105                 110

cat ggg ttt gga tct gcc ttc ctc tca gtg caa gac agt att gga gag      442
His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Asp Ser Ile Gly Glu
115                 120                 125                 130

gaa ggc cca gca ctg ccg acc tgg gcc atc atc ctg ctg gca gtg gcc      490
Glu Gly Pro Ala Leu Pro Thr Trp Ala Ile Ile Leu Leu Ala Val Ala
                135                 140                 145

ttt tcc ttg ctc ttg atc ctg atc att gtt ttg att ata ata ttc tgt      538
Phe Ser Leu Leu Leu Ile Leu Ile Ile Val Leu Ile Ile Ile Phe Cys
            150                 155                 160

tgc tgt tgt gcc tcc agg aga gaa aag gaa gaa tct act tat caa aat      586
Cys Cys Cys Ala Ser Arg Arg Glu Lys Glu Glu Ser Thr Tyr Gln Asn
```

```
        165                 170                 175

gaa ata agg aaa tct gca aac atg agg aca aac aaa gca gat ccg gag      634
Glu Ile Arg Lys Ser Ala Asn Met Arg Thr Asn Lys Ala Asp Pro Glu
    180                 185                 190

aca aag tta aaa agt gga aag gaa aac tac ggg tac agt tcg gat gag      682
Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr Gly Tyr Ser Ser Asp Glu
195                 200                 205                 210

gca aag gct gca cag act gca tct ctc cct cct aaa tct gct gaa gtc      730
Ala Lys Ala Ala Gln Thr Ala Ser Leu Pro Pro Lys Ser Ala Glu Val
                215                 220                 225

agc ctt cca gaa aaa cgc agc agt agc ctt cct tat cag gaa ctc aat      778
Ser Leu Pro Glu Lys Arg Ser Ser Ser Leu Pro Tyr Gln Glu Leu Asn
            230                 235                 240

aaa cat cag ccc ggt cca gca act cat cca cgg gtt tcc ttt gac atc      826
Lys His Gln Pro Gly Pro Ala Thr His Pro Arg Val Ser Phe Asp Ile
        245                 250                 255

gcc agt cct cag aag gtc aga aat gtg act tta gtg taataaagac           872
Ala Ser Pro Gln Lys Val Arg Asn Val Thr Leu Val
    260                 265                 270

ttctcatgac tgtacttggt gca                                           895
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<210> 12
<211> 270
<212> PRT
<213> Mus musculus

<400> 12

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Met Glu Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala
1               5                   10                  15

Gln Leu Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln
            20                  25                  30

Asn Val Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val
        35                  40                  45

Thr His Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val
    50                  55                  60

Leu Ser Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Thr
65                  70                  75                  80

Tyr Ala Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile
                85                  90                  95

His Asp Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln
            100                 105                 110
```

```
Asn Ser His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Asp Ser Ile
        115                 120                 125

Gly Glu Glu Gly Pro Ala Leu Pro Thr Trp Ala Ile Ile Leu Leu Ala
    130                 135                 140

Val Ala Phe Ser Leu Leu Leu Ile Leu Ile Ile Val Leu Ile Ile Ile
145                 150                 155                 160

Phe Cys Cys Cys Cys Ala Ser Arg Arg Glu Lys Glu Glu Ser Thr Tyr
                165                 170                 175

Gln Asn Glu Ile Arg Lys Ser Ala Asn Met Arg Thr Asn Lys Ala Asp
            180                 185                 190

Pro Glu Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr Gly Tyr Ser Ser
        195                 200                 205

Asp Glu Ala Lys Ala Ala Gln Thr Ala Ser Leu Pro Pro Lys Ser Ala
    210                 215                 220

Glu Val Ser Leu Pro Glu Lys Arg Ser Ser Ser Leu Pro Tyr Gln Glu
225                 230                 235                 240

Leu Asn Lys His Gln Pro Gly Pro Ala Thr His Pro Arg Val Ser Phe
                245                 250                 255

Asp Ile Ala Ser Pro Gln Lys Val Arg Asn Val Thr Leu Val
            260                 265                 270
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<210> 13
<211> 754
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (53)..(721)

<400> 13

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gtgaacgaga tacagagatt tacctgcctg aggtaaggaa gatcatgctg ag atg gag      58
                                                          Met Glu
                                                          1

ggc agc tgg aga gat gtc ctg gct gtg ctg gtc atc ctg gct cag ctg      106
Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala Gln Leu
        5                   10                  15

aca gct tcc gga tcc agt tat cag atc ata gaa ggt cct cag aat gta      154
Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln Asn Val
    20                  25                  30

aca gtc cta aag gac tca gag gct cac ttc aac tgc acc gtg act cac      202
Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val Thr His
```

```
35                  40                  45                  50

ggc tgg aag ctt ctc atg tgg act ctt aac caa atg gtg gtg ctg agt      250
Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val Leu Ser
                55                  60                  65

ctc acc acc caa gga ccc atc atc acc aac aac cgc ttc acc tat gcc      298
Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Thr Tyr Ala
            70                  75                  80

agt tac aac agc act gac agc ttc atc tcg gag ttg atc atc cat gat      346
Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile His Asp
        85                  90                  95

gtg cag ccc agt gac tcg gga tcc gtg caa tgc agc ctg cag aac agc      394
Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln Asn Ser
    100                 105                 110

cat ggg ttt gga tct gcc ttc ctc tca gtg caa gaa tct act tat caa      442
His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Glu Ser Thr Tyr Gln
115                 120                 125                 130

aat gaa ata agg aaa tct gca aac atg agg aca aac aaa gca gat ccg      490
Asn Glu Ile Arg Lys Ser Ala Asn Met Arg Thr Asn Lys Ala Asp Pro
                135                 140                 145

gag aca aag tta aaa agt gga aag gaa aac tac ggg tac agt tcg gat      538
Glu Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr Gly Tyr Ser Ser Asp
            150                 155                 160

gag gca aag gct gca cag act gca tct ctc cct cct aaa tct gct gaa      586
Glu Ala Lys Ala Ala Gln Thr Ala Ser Leu Pro Pro Lys Ser Ala Glu
        165                 170                 175

gtc agc ctt cca gaa aaa cgc agc agt agc ctt cct tat cag gaa ctc      634
Val Ser Leu Pro Glu Lys Arg Ser Ser Ser Leu Pro Tyr Gln Glu Leu
    180                 185                 190

aat aaa cat cag ccc ggt cca gca act cat cca cgg gtt tcc ttt gac      682
Asn Lys His Gln Pro Gly Pro Ala Thr His Pro Arg Val Ser Phe Asp
195                 200                 205                 210

atc gcc agt cct cag aag gtc aga aat gtg act tta gtg taataaagac      731
Ile Ala Ser Pro Gln Lys Val Arg Asn Val Thr Leu Val
                215                 220

ttctcatgac tgtacttggt gca                                          754
```

<210> 14
<211> 223
<212> PRT
<213> Mus musculus

<400> 14

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Met Glu Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala
1               5                   10                  15

Gln Leu Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln
            20                  25                  30
```

```
Asn Val Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val
        35                  40                  45

Thr His Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val
    50                  55                  60

Leu Ser Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Thr
65                  70                  75                  80

Tyr Ala Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile
                85                  90                  95

His Asp Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln
            100                 105                 110

Asn Ser His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Glu Ser Thr
        115                 120                 125

Tyr Gln Asn Glu Ile Arg Lys Ser Ala Asn Met Arg Thr Asn Lys Ala
    130                 135                 140

Asp Pro Glu Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr Gly Tyr Ser
145                 150                 155                 160

Ser Asp Glu Ala Lys Ala Ala Gln Thr Ala Ser Leu Pro Pro Lys Ser
                165                 170                 175

Ala Glu Val Ser Leu Pro Glu Lys Arg Ser Ser Ser Leu Pro Tyr Gln
            180                 185                 190

Glu Leu Asn Lys His Gln Pro Gly Pro Ala Thr His Pro Arg Val Ser
        195                 200                 205

Phe Asp Ile Ala Ser Pro Gln Lys Val Arg Asn Val Thr Leu Val
    210                 215                 220
```

<210> 15
<211> 631
<212> PRT
<213> Rattus rattus

<400> 15

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Met Glu Gly Ser Trp Arg Asp Val Leu Ala Val Leu Val Ile Leu Ala
1               5                   10                  15

Gln Leu Thr Ala Ser Gly Ser Ser Tyr Gln Ile Ile Glu Gly Pro Gln
            20                  25                  30

Met Ala Tyr Ser Cys Gln Pro Leu Gln Glu Ser Pro Leu Leu Gly Phe
        35                  40                  45
```

Pro Arg Leu Arg Phe Ile His Leu Phe Val Leu Leu Val Gly Leu
50 55 60

Leu Gln Ile Ser Ser Gly Ile Val Gly Gln Val Ser Lys Ser Val Arg
65 70 75 80

Asn Val Thr Val Leu Lys Asp Ser Glu Ala His Phe Asn Cys Thr Val
85 90 95

Thr His Gly Trp Lys Leu Leu Met Trp Thr Leu Asn Gln Met Val Val
100 105 110

Leu Ser Leu Thr Thr Gln Gly Pro Ile Ile Thr Asn Asn Arg Phe Glu
115 120 125

Lys Ala Leu Leu Ser Cys Asp Tyr Lys Phe Cys Ser Glu Glu Gln Ser
130 135 140

Ile His Arg Ile Tyr Trp Gln Lys His Asp Lys Met Val Leu Ser Val
145 150 155 160

Ile Ser Gly Val Pro Glu Val Trp Pro Lys Tyr Lys Asn Arg Thr Thr
165 170 175

Tyr Ala Ser Tyr Asn Ser Thr Asp Ser Phe Ile Ser Glu Leu Ile Ile
180 185 190

His Asp Val Gln Pro Ser Asp Ser Gly Ser Val Gln Cys Ser Leu Gln
195 200 205

Asn Ser His Gly Phe Gly Ser Ala Phe Leu Ser Val Gln Val Tyr Asp
210 215 220

Ile Ala Asn Asn Tyr Ser Phe Ser Leu Leu Gly Leu Ile Leu Ser Asp
225 230 235 240

Arg Gly Thr Tyr Thr Cys Val Val Gln Arg Tyr Glu Gly Gly Ser Tyr
245 250 255

Val Val Lys His Leu Thr Thr Val Glu Val Met Gly Thr Leu Asn Ile
260 265 270

Pro Ser Asn Asn Leu Ile Val Thr Glu Gly Glu Pro Cys Asn Val Thr
275 280 285

Cys Tyr Ala Val Gly Trp Thr Ser Leu Pro Asp Ile Ser Trp Glu Leu
290 295 300

Glu Val Pro Val Ser His Ser Leu Ser Val Arg Ala Asp Phe Pro Thr
305 310 315 320

Pro Asn Ile Thr Glu Tyr Gly Asn Pro Ser Ala Asp Ile Lys Arg Ile
325 330 335

Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Leu Ser Trp Leu
340 345 350

Glu Asn Gly Arg Glu Leu Asn Ser Tyr Asn Ser Phe Leu Glu Pro Gly
355 360 365

Asn Phe Met Arg Val Leu Ser Val Leu Asp Leu Thr Pro Leu Gly Asn
370 375 380

Gly Thr Leu Thr Cys Val Ala Glu Leu Lys Asp Leu Gln Ala Ser Lys

385 390 395 400

Ser Leu Thr Val Asn Leu Gly Ile Asn Thr Thr Ile Ser Gln Asp Pro
405 410 415

Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp Phe Asn Ala Thr
420 425 430

Tyr Asp His Phe Ile Asp Cys Phe Ile Glu Tyr Gly Asp Ala His Val
435 440 445

Ser Gln Asn Phe Thr Val Val Gln Pro Pro Pro Asp Ser Ile Gly Glu
450 455 460

Glu Gly Pro Ala Leu Pro Thr Trp Ala Ile Ile Leu Leu Ala Val Ala
465 470 475 480

Phe Ser Leu Leu Leu Ile Leu Ile Ile Val Leu Ile Ile Ile Phe Thr
485 490 495

Trp Val Lys Pro Pro Glu Asp Pro Pro Asp Glu Lys Gln Thr Val Pro
500 505 510

Phe Ala Trp Ala Gly Pro Asp Ala Val Lys Ala Ile Ile Ile Phe Phe
515 520 525

Ile Ala Ile Thr Val Ile Ala Val Ile Ala Ala Ile Ala Ile Ile Ile
530 535 540

Phe Cys Cys Cys Cys Ala Ser Arg Arg Glu Lys Glu Glu Ser Thr Tyr
545 550 555 560

Gln Asn Glu Ile Arg Lys Ser Ala Asn Met Arg Thr Asn Lys Ala Asp
565 570 575

Pro Glu Thr Lys Leu Lys Ser Gly Lys Glu Asn Tyr Gly Tyr Ser Ser
580 585 590

Asp Glu Cys Ile Thr Val Lys Phe Arg Arg Cys Phe Arg Arg Arg Asn
595 600 605

Glu Ala Ser Arg Glu Thr Asn Lys Asn Leu Tyr Ile Gly Pro Val Glu
610 615 620

Ala Ala Ala Glu Gln Thr Val
625 630